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(54) Immunosuppressant able to block the interleukin - 2 response.

(57) Provided is a polypeptide which specifically binds to the γ-chain of human interleukin-2 receptor to selectively inhibit the binding of the γ-chain of human interleukin-2 receptor to the β-chain of the same, therefore having an activity of blocking the human interleukin-2 response. Also provided are an immunosuppressant containing the polypeptide, a DNA gene coding for the polypeptide, a recombinant DNA having the gene, a transformant having the recombinant DNA, and a method for producing the objective polypeptide by incubating the transformant. The novel polypeptide is usable, independently or along with substances capable of inhibiting the binding of interleukin-2 to interleukin-2 receptor, as a medicine effective in preventing the rejection of grafts after transplantation and also in curing inflammatory diseases such as allergic diseases and autoimmune diseases, the possibility that interleukin-2 will actively participate in the rejection and also in such inflammatory diseases having been suggested.

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FIELD OF THE INVENTION

The present invention relates to a polypeptide which specifically binds to the γ-chain of the interleukin-2 receptor to selectively inhibit the binding of the γ-chain of the interleukin-2 receptor to the β-chain of the same, therefore being able to block the interleukin-2 response, and also to a pharmaceutical composition containing the polypeptide, a DNA coding for the polypeptide, a recombinant DNA containing the gene, a transformant bearing the recombinant DNA, and a method for producing the polypeptide by cultivating the transformant.

The polypeptide of the present invention is a valuable substance which is usable, independently or along with substances capable of inhibiting the binding of interleukin-2 to interleukin-2 receptor, as a medicine effective in preventing the rejection after transplantation and also in curing inflammatory diseases such as allergic diseases and autoimmune diseases, the possibility that interleukin-2 will participate in the rejection and also in such inflammatory diseases having been suggested.

"Interleukin-2" may be hereinafter referred to as "IL-2".

BACKGROUND OF THE INVENTION

In these days when the surgical technique for transplantation has been improved noticeably, the success in transplantation essentially depends upon how to inhibit the rejection of the grafts after the operation. The rejection results from a series of immunoreactions occurring through the interaction of the grafts with organs of recipients which have recognized the grafts as foreign matter to reject them. Therefore, so-called immunosuppressants such as various steroids, azathiopurine, methotrexate and 6-mercaptopurine have heretofore been used as rejection-inhibiting medicines. However, since the safety range of such medicines was narrow and their effects were weak, extreme improvement in the take of grafts in recipients could not be attained even though such medicines were applied to them.

Using cyclosporin A that has been developed recently, the take of grafts in recipients has become improved surprisingly. However, it has been clarified that cyclosporin A has serious nephrotoxicity, which has obliged us to limit its use.

Given the situations, it is desired to develop more safe, more specific and more effective immunosuppressants.

IL-2 is a protein that is produced by helper T-cells, and it is an extremely important factor for host defence machinery, as having various functions of a broad range, for example, for induction of proliferation and differentiation of killer T-cells and induction of differentiation of B cells in living bodies. It has been said that killer T-cells activated by IL-2, etc. will participate very much in the host vs. graft reaction (HVG reaction) or the graft vs. host reaction (GVH reaction) which is considered to be the key to the take of grafts in transplantation of organs or bone marrow.

On the other hand, it is considered that autoimmune diseases will be caused by an imbalance of the immune system in a living body, resulting in attacking the body itself. In particular, there is a high possibility that excess production of factors participating in the immune system, such as IL-2, as well as excess reaction thereto will essentially cause an imbalance of the immune system.

Given the situations, it has been considered that if the IL-2 response may be selectively and effectively inhibited, then the rejection after transplantation may be prevented and autoimmune diseases may be cured. In fact, there have been published reports in which IL-2 and a cytotoxin were fused together to prepare a polypeptide capable of selectively damaging IL-2 responding cells having an IL-2 receptor and the polypeptide was administered to a rat with adjuvant arthritis, which is one of the animal models with an autoimmune disease, with the result that the onset of the symptoms of arthritis was delayed in the rat and the presented symptoms of arthritis in the same were slight. The report further revealed that when the polypeptide was administered to a mouse that had received a cardiac graft by transplantation from an allogenic mouse, during the transplantation, then the rejection against the transplanted cardiac graft in the recipient mouse was inhibited. (See Proc. Natl. Acad. Sci. USA, Vol. 86, page 1008, 1989.)

However, the polypeptide to be obtained by fusing IL-2 and a cytotoxin has a short half-life period in blood so that a large amount of the polypeptide must be dosed in order to attain the intended effect, which, however, will cause some harmful side effects. Given the situations, the development of a medicine which is safer, more specific and more effective in inhibiting the IL-2 response has been desired.

Heretofore, it has been known that the IL-2 receptor on IL-2 responding cells is composed of two glycoprotein molecules, one being an α-chain having a molecular weight of about 55 kd and the other being a β-chain having a molecular weight of about 75 kd. The dissociation constant of the binding between the respective molecules and IL-2 is 10^{-8} M for the α-chain and 10^{-9} M for the β-chain. It has been considered

that, when both the α-chain and the β-chain are bound to IL-2 to at the same time, then the resulting associate will have high-affinity binding with a dissociation constant of 10^{-12} M.

However, it has been clarified that, even when a human β-chain cDNA is transfected into mouse non-lymphocytic cells, it does not bind to IL-2 and that, even when both human α-chain and β-chain cDNA's are transfected into the same, they may bind to IL-2 only to intermediate degree without forming any high-affinity binding to the same. (See Science, Vol. 244, page 551, 1989.) Hence, the existence of another different third molecule than the α-chain and the β-chain, which is crucical for the binding to IL-2, has become suggested.

We, the present inventors have cloned a gene coding for a glycoprotein molecule having a molecular weight of 64 kd, which is the third molecule constituting IL-2 receptor complex (hereinafter referred to as IL-2 receptor γ-chain) (see Japanese Patent Application No. 4-104947 and Science, Vol. 257, page 379, 1992), by which the IL-2/IL-2 receptor system has been clarified completely.

Precisely, it has been clarified that the analysis of an associate formed by transfection of both human β-chain and γ-chain cDNA's into mouse non-lymphocytic cells shows the binding to intermediate degree between them, which, however, has previously been considered to be caused by the β-chain only, and that the transfection of all human α-chain, β-chain and γ-chain cDNA's into the same gives high-affinity binding between them, which, however has previously been considered to be caused by the α-chain and the β-chain only. In addition, it has been shown that the internalization of an IL-2/IL-2 receptor complex into cells, which is considered to be a prerequisite for signal transduction for IL-2, does not occur in the cells expressing only β-chain or in the cells expressing both α-chain and β-chain but may occur in the cells additionally expressing the γ-chain. Accordingly, it has been clarified that the IL-2 receptor γ-chain molecule, of which the finding the present inventors have been succeeded in for the first time in the world, is not a molecule which merely defines its binding to IL-2 but a molecule which is indispensable for the signal transduction triggered by IL-2.

It has been known that the IL-2 receptor γ-chain molecule binds to the extracellular site in IL-2 receptor β-chain in the presence of IL-2. (See Science, Vol. 257, page 379, 1992.) Therefore, it is considered that if the binding between the β-chain and the γ-chain is inhibited, the β-chain only could not form its binding itself to IL-2 while both the α-chain and the β-chain may form their binding to IL-2 merely to some intermediate degree but the IL-2-signal transduction will be blocked. Namely, if the binding between the IL-2 receptor β-chain and the IL-2 receptor γ-chain on IL-2 responding cells can be inhibited, the IL-2 signal transduction may be completely blocked and the cells will become unresponsive to IL-2. By the inhibition, therefore, the rejection during transplantation, in which IL-2 is considered to participate, may be prevented and autoimmune diseases, in which IL-2 is considered to also actively participate, may be cured. Up to the present, however, a substance having an activity of inhibiting the binding between the IL-2 receptor β-chain and the IL-2 receptor γ-chain is not known at all.

SUMMARY OF THE INVENTION

Accordingly, the object of the present invention is to provide a polypeptide which specifically binds to the γ-chain of the IL-2 receptor to selectively inhibit the binding of the γ-chain of the IL-2 receptor to the β-chain of the same, therefore being able to block the IL-2 response, and also a pharmaceutical composition containing the polypeptide, a DNA coding for the polypeptide, a recombinant DNA having the DNA, a transformant having the recombinant DNA, and a method for producing the objective polypeptide by cultivating the transformant.

The polypeptide of the present invention is a valuable substance which is usable, independently or along with substances capable of inhibiting the binding of IL-2 to the IL-2 receptor, as a medicine effective in preventing the rejection of grafts after transplantation and also in curing inflammatory diseases such as allergic diseases and autoimmune diseases, the possibility that IL-2 will participate in the rejection and also in such inflammatory diseases having been suggested.

We, the present inventors have repeated assiduous studies so as to attain the above-mentioned object and, as a result, have found that the objective polypeptide, which specifically binds to the γ-chain of human IL-2 receptor selectively inhibit the binding of the γ-chain of the IL-2 receptor to the β-chain thereof, therefore being able to block the IL-2 response, in accordance with the process mentioned below. On the basis of the finding, we have completed the present invention.

Accordingly, the present invention provides a polypeptide which specifically binds to the γ-chain of the IL-2 receptor, preferably the human IL-2 receptor to selectively inhibit the binding of the γ-chain of the IL-2 receptor to the β-chain thereof, therefore being able to block the IL-2 response, and also a pharmaceutical composition containing the polypeptide, a DNA coding for the polypeptide, a recombinant DNA containing

the DNA, a transformant bearing the recombinant DNA, and a method for producing the objective polypeptide by cultivating the transformant.

First, there is provided in accordance with the present invention a polypeptide which specifically binds to the γ-chain of interleukin-2 receptor, in a preferred embodiment to the γ-chain of the human interleukin-2 receptor and being able to block the IL-2 response.

In a preferred embodiment, the polypeptide is a monoclonal antibody, which is preferably derived from mouse.

As another preferred embodiment, the monoclonal antibody is produced by cells of GP-2, GP-4 or AG14.

As still another preferred embodiment, the monoclonal antibody is produced by cells of TUGh4, TUGh5 or AG14.

As still another preferred embodiment, the polypeptide contains only the variable region of the monoclonal antibody.

As still another preferred embodiment, the polypeptide has the following amino acid sequence (I).

```
ATG GAC ATC CTG CTG ACC CAG TCT CCA TCA ATC ATG TCT GCA TCT CTA   48
Met Asp Ile Leu Leu Thr Gln Ser Pro Ser Ile Met Ser Ala Ser Leu
 1               5                  10                  15

GGG GAA CGG GTC ACC ATG ACC TGC ACT GCC AGC TCA AGT GTA AGT TCC   96
Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser
            20                  25                  30

AGT TAC TTG CAC TGG TAC CAG CAG AAG CCA GGA TCC TCC CCC AAA CTC  144
Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu
        35                  40                  45

TGG ATT TAT AGC ACA TCC AAC CTG GCT TCT GGA GTC CCA GCT CGC TTC  192
Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe
    50                  55                  60

AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC AGC ATG  240
Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met
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65                  70                  75                  80
GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAC CAG TAT CAT CGT TCC  288
Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser
                85                  90                  95
CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTC AAA GTC GAC AAA  336
Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Val Asp Lys
            100                 105                 110
TCC TCA GGA TCT GGC TCC GAA TCC AAA AGC ACG CAG GTC AAA CTC GAG  384
Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln Val Lys Leu Glu
        115                 120                 125
GAG TCT GGA TCT GAG CTG GTG AGG CCT GGA GCT TCA GTG AAG CTG TCC  432
Glu Ser Gly Ser Glu Leu Val Arg Pro Gly Ala Ser Val Lys Leu Ser
    130                 135                 140
TGC AAG GCT TCT GGC TAC ACA TTC ACC AGC TAC TGG ATG CAC TGG GTG  480
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met His Trp Val
145                 150                 155                 160
AAG CAG AGG CAT GGA CAA GGC CTT GAG TGG ATT GGA AAT ATT TAT CCT  528
Lys Gln Arg His Gly Gln Gly Leu Glu Trp Ile Gly Asn Ile Tyr Pro
                165                 170                 175
GGT AGT GGT AGT ACT AAC TAC GAT GAG AAG TTC AAG AGC AAG GCC ACA  576
Gly Ser Gly Ser Thr Asn Tyr Asp Glu Lys Phe Lys Ser Lys Ala Thr
            180                 185                 190
CTG ACT GTA GAC ACA TCC TCC AGC ACA GCC TAC ATG CAC CTC AGC AGC  624
Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr Met His Leu Ser Ser
        195                 200                 205
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CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT ACA AGA AGC AGC CGG  672
Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Thr Arg Ser Ser Arg
    210                 215                 220
AAC TGG GTC TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC  720
Asn Trp Val Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
225                 230                 235                 240
ACC GTC TCC TCA                                              732
Thr Val Ser Ser
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As still another preferred embodiment, the polypeptide has the following amino acid sequence (II).

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ATG GAT ATT CTG CTG ACA CAG TCT CCA GCC TCC CTA TCT GCA TCT GTG   48
Met Asp Ile Leu Leu Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val
 1               5                  10                  15
GGA GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GGG AAT ATT CAC AAT   96
Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn
            20                  25                  30
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TAT TTA GCA TGG TAT CAG CAG AAA CAG GGA AAA TCT CCT CAG CTC CTG  144
Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu
        35                  40                  45
GTC TAT AAT GCA AAA ACC TTA GCA GAT GGT GTG CCA TCA AGG TTC AGT  192
Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser
    50                  55                  60
GGC AGT GGA TCA GGA ACA CAA TAT TCT CTC AAG ATC AAC AGC CTG CAG  240
Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln
65                  70                  75                  80
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CCT GAA GAT TTT GGG AGT TAT TAC TGT CAA CAT TTT TGG AGT ACT CCG  288
Pro Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro
                 85                  90                  95

TGG ACG TTC GGT GGA GGG ACC AAG CTG GAG CTC AAA GTC GAG AAA TCC  336
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Val Asp Lys Ser
            100                 105                 110

TCA GGA TCT GGC TCC GAA TCC AAA AGC ACG CAG GTC AAA CTC GAG GAG  384
Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln Val Lys Leu Glu Glu
        115                 120                 125

TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT TCA GTG AAG ATA TCC TGC  432
Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
    130                 135                 140

AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC TAC ATG CAC TGG GTG AAG  480
Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Tyr Met His Trp Val Lys
145                 150                 155                 160

CAA AGC CAT GTA AAG AGC CTT GAG TGG ATT GGA CGT ATT AAT CCT TAC  528
                                    60

Gln Ser His Val Lys Ser Leu Glu Trp Ile Gly Arg Ile Asn Pro Tyr
                165                 170                 175

AAT GGT GCT ACT AGC TAC AAC CAG AAT TTC AAG GAC AAG GCC AGC TTG  576
Asn Gly Ala Thr Ser Tyr Asn Gln Asn Phe Lys Asp Lys Ala Ser Leu
            180                 185                 190

ACT GTA GAT AAG TCC TCC AGC ACA GCC TAC ATG GAG CTC CAC AGC CTG  624
Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu His Ser Leu
        195                 200                 205

ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA GAG AAT TAC TAC  672
Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Tyr
    210                 215                 220

GGT AGT AGC TAC GGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT  720
Gly Ser Ser Tyr Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240

GTC TCT GCA                                                     729
Val Ser Ala
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In still another preferred embodiment the polypeptide has an amino acid of Sequence No. (I) or (II) mentioned above, from which its N-terminal Met has been deleted.

As still another preferred embodiment, the polypeptide has an amino acid sequence of Sequence No. (I), (1) of which a part has been deleted, (2) of which a part has been substituted by a different amino acid sequence, (3) to which amino acid residue(s) or peptide(s) has/have been added, or (4) of which a part has

been acetylated, amidated or modified with polyethylene glycol(s).

As still another preferred embodiment, the polypeptide has an amino acid sequence of Sequence No. (II), (1) of which a part has been deleted, (2) of which a part has been substituted by a different amino acid sequence, (3) to which amino acid residue(s) or peptide(s) has/have been added, or (4) of which a part has been acetylated, amidated or modified with polyethylene glycol(s).

As still another preferred embodiment, the polypeptide is such that the constant region of the monoclonal antibody has been changed into the constant region of an antibody of another species, preferably human.

As still another preferred embodiment, the polypeptide is such that the constant region and the frame-work sequence of the variable region of the mouse monoclonal antibody have been changed into the constant region and the frame-work sequence of a human antibody, respectively.

Secondly, also provided is a pharmaceutical composition, preferably an immunosuppressant containing the novel polypeptide.

As one preferred embodiment, the pharmaceutical composition contains the novel polypeptide along with an anti-interleukin-2 receptor α-chain antibody and/or an anti-interleukin-2 receptor β-chain antibody, which antibodies are preferably directed against the human α- and β-chains.

As another preferred embodiment, the pharmaceutical composition or immunosuppressant, respectively, contains the novel polypeptide along with (a) a polypeptide containing the variable region of an anti-human interleukin-2 receptor α-chain antibody, a polypeptide derived from the polypeptide by deleting a part of it, a polypeptide derived from the polypeptide by substituting a part of it by other amino acid(s), or a polypeptide derived from the polypeptide by adding other amino acid residue(s), other polypeptide(s) or other substance(s) thereto, and/or (b) a polypeptide containing the variable region of an anti-human interleukin-2 receptor β-chain antibody, a polypeptide derived from the polypeptide by deleting a part of it, a polypeptide derived from the polypeptide by substituting a part of it by other amino acid(s), or a polypeptide derived from the polypeptide by adding other amino acid residue(s), other polypeptide(s) or other substance(s) thereto.

As still another preferred embodiment, the pharmaceutical composition contains the novel polypeptide along with an anti-human interleukin-2 receptor α-chain antibody containing the human constant region and/or an anti-human interleukin-2 receptor β-chain antibody containing the human constant region.

As still another preferred embodiment, the pharmaceutical composition contains the novel polypeptide along with an anti-human interleukin-2 receptor α-chain antibody containing the human constant region and the frame-work sequence of the human variable region and/or an anti-human interleukin-2 receptor β-chain antibody containing the human constant region and the frame-work sequence of the human variable region.

Thirdly, also provided is a DNA coding for the novel polypeptide.

As one preferred embodiment, the DNA has a nucleotide sequence as shown under Sequence No. (I).

As another preferred embodiment, the DNA has a nucleotide sequence as shown under Sequence No. (II).

The above illustrated DNA-sequences may furthermore be selected from a DNA-sequence, which in respect to (I) or (II) is deficient in one or more nucleotides; in which in respect to (I) or (II) one or more nucleotides are replaced; and a DNA-sequence, to which in respect to (I) or (II) one or more nucleotides are added.

Fourthly, also provided is a recombinant DNA containing the novel DNA.

Fifthly, also provided is a transformant bearing the novel recombinant DNA.

As one preferred embodiment, the transformant is a procaryote preferably Escherichia coli or an eucaryotic cell.

Sixthly, also provided is a method of producing the novel polypeptide by cultivating the novel transformant to produce the polypeptide followed by collecting the polypeptide.

BRIEF EXPLANATION OF THE DRAWINGS

Fig. 1 shows a process of constructing plasmid pIL-2RGS.

Fig. 2 shows the sequences of primers used in Example 3.

Fig. 3 shows the DNA sequence of the linker used in Example 4 for linking the V-region of the L-chain and the V-region of the H-chain.

Fig. 4 shows a process of constructing plasmid pFv-DE.

Fig. 5 shows a process of constructing plasmid pFv(GP-2) and plasmid pFv(GP-4).

Fig. 6 shows the activities of monoclonal antibodies GP-2 and GP-4 and polypeptides Fv(GP-2) and Fv-(GP-4) which inhibit the IL-2-dependent growth of ILT-Mat cells, wherein Δ indicates the activity of a control

antibody, □ indicates the activity of GP-2, □ indicates the activity of Fv(GP-2), ○ indicates the activity of GP-4, and ○ indicates the activity of Fv(GP-4).

Fig. 7 shows the activity of the monoclonal antibody GP-2 which inhibits the IL-2-dependent growth of ILT-Mat cells in the presence of an anti-IL-2 receptor α-chain antibody (H31) and/or anti-IL-2 receptor β-chain antibody (TU25), wherein △ indicates the activity of a control antibody, ▣ indicates the activity of GP-2, □ indicates the activity of anti-IL-2R β-chain antibody, △ indicates the activity of GP-2 in the presence of anti-IL-2R β-chain antibody, ○ indicates the activity of anti-IL-2R α-chain antibody, ○ indicates the activity of GP-2 in the presence of anti-IL-2R α-chain antibody, and ◇ indicates the activity of GP-2 in the presence of both anti-IL-2R α-chain antibody and anti-IL-2R α-chain antibody.

Fig. 8 shows the activity of monoclonal antibody TUGm2 which inhibits the IL-2-dependent growth of CTLL-2 cells by itself or in the presence of anti-mouse IL-2 receptor β-chain antibody, wherein ◎ indicates the activity of a control sample to which no antibody was added, ○ indicates the activity of TUGm2, □ indicates the activity of the anti-IL-2R β-chain antibody, and □ indicates the activity of TUGm2 in the presence of anti-IL-2R β-chain antibody.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a polypeptide which specifically binds to the γ-chain of the IL-2 receptor to selectively inhibit the binding of the γ-chain of human IL-2 receptor to the β-chain thereof, therefore being able to block the IL-2 response.

We, the present inventors have first produced a number of hybridomas capable of producing a mouse monoclonal antibody that specifically binds to the γ-chain of human IL-2 receptor, from which we have selected the hybridoma clone that produces a mouse monoclonal antibody having the activity of inhibiting the IL-2 response. A method of preparing the hybridoma clone that produces a mouse anti-human IL-2 receptor γ-chain monoclonal antibody will be mentioned below.

The hybridomas are produced by fusing myeloma cells and antibody-producing cells. As the antibody-producing cells, usable are cells of the spleen or the lymph node of an animal, such as mouse or rat, that has been immunized with a recombinant human IL-2 receptor γ-chain molecule. As the immunizing substance, usable are single, recombinant human IL-2 receptor molecules, fused molecules composed of the molecule and other protein(s), and polypeptides which are a part of the molecule. Above all, recombinant human IL-2 receptor molecules having only the extracellular region are especially efficiently used. In place of the recombinant human IL-2 receptor γ-chain molecules, also usable are human cells expressing a human IL-2 receptor γ-chain molecule as well as mouse cells, etc. into which a gene coding for a human IL-2 receptor γ-chain molecule has been transfected to be able to produce the γ-chain molecule therein by biosynthesis. In addition, also usable are the γ-chain itself that has been purified from such cells, as the immunogen.

The species of the animals from which the antibody-producing cells and the myeloma cells are derived may be different from each other, provided that both cells may be fused together, but in general, the both cells derived from an animal of one and the same species are preferably fused to obtain a favorable result. As one preferred mode for carrying out the present invention, used are hybridomas to be obtained by fusing cells of the spleen or lymph node of a mouse that has been immunized with a polypeptide having only the extracellular region of a human recombinant IL-2 receptor γ-chain molecule and mouse myeloma cells.

As one example, mentioned are hybridomas obtained by fusing cells of the spleen of a Balb/c mouse that has been immunized with a polypeptide having only the extracellular region of a human recombinant IL-2 receptor γ-chain molecule, as suspended in a physiological saline solution, and myeloma cells SP2/0-Ag14 of a Balb/c mouse. Using the hybridomas, an excellent result has been obtained, as shown in the example mentioned below.

The polypeptide having only the extracellular region of a human recombinant IL-2 receptor γ-chain molecule may be obtained by cultivating a transformant having an expression plasmid vector containing a gene coding for the molecule. As the transformant, usable is any of procaryotic cells such as E. coli, etc. as well as eucaryotic cells such as CHO cells, etc.

As the myeloma cells, also usable, in addition to SP2/0-Ag14, are those from 8-azaguanine-resistant cell strains, such as mouse myeloma cells X63-Ag8-6.5.3, P3-X63-Ag8-U1, P3-X63-Ag8, P3-NSI/1-Ag4-1 and MPC11-4.5.6.TG.1.7, rat myeloma cells 210.RCY.Ag1.2.3, human myeloma cells SKO-007 and GH15006TG-A12, etc. The formation of the hybridomas as well as the selection of the hybridoma clone which produces a monoclonal antibody capable of binding to the γ-chain molecule of IL-2 receptor and therefore having an activity of blocking the IL-2 response, from among them, may be conducted, for example, as mentioned

below. First, the antibody-producing cells and the myeloma cells are fused together, using polyethylene glycol or Sendai viruses, etc. Only the fused hybridomas may grow in a medium containing hypoxanthine, thymidine and aminopterin (HAT medium). All the thus-obtained hybridoma do not always produce antibodies and all the antibody-producing hybridomas do not always produce the objective antibody. Therefore, a hybridoma clone that produces a monoclonal antibody capable of binding to the γ-chain molecule of IL-2 receptor and therefore having an activity of blocking the IL-2 response must be selected from among them.

The selection may be conducted, for example, according to the process mentioned below. Precisely, the binding of the antibody that has been produced in the supernatant of the hybridoma culture to mouse L929 cells into which a gene coding for a human IL-2 receptor γ-chain molecule has been transfected and which therefore express the peptide (hereinafter referred to as Lγ cells) and the binding of the same to mouse L929 cells into which a gene coding for a human IL-2 receptor β-chain molecule has been transfected and which therefore express the peptide (hereinafter referred to as Lβ cells) are measured, and such that the binding of the antibody to the former is high while that to the latter is low is selected. The hybridoma thus selected to produce the antibody shall be the hybridoma that produces the antibody capable of specifically binding to the γ-chain molecule of IL-2 receptor.

For the measurement of the binding of the antibody to the cells, any of radioimmunoassay using a radioisotope-labelled anti-mouse immunoglobulin antibody, fluorescent immunoassay using a fluorescent dye-labelled anti-mouse immunoglobulin antibody, etc. As the cells to be used for the screening, any combination comprised of cells that express the human γ-chain and cells that do not express the same may be employed.

Since not all the antibodies capable of specifically binding to the γ-chain molecule of the IL-2 receptor do have the capability of blocking the IL-2 response, hybridomas that produce an antibody having the activity of blocking the IL-2 response are selected from among those that produce the antibody to the γ-chain molecule of IL-2 receptor, in accordance with the process mentioned below.

Precisely, the supernatant of the hybridoma culture is added to ILT-Mat cells of a human adult T-cell leukemia virus-infected T-cell line having a human IL-2-dependent growth activity, whereupon the growth-inhibiting activity of the hybridomas, if any, against the ILT-Mat cells is measured. (See Journal of Experimental Medicine, Vol. 169, page 1323. 1989) If the hybridomas show the growth-inhibiting activity, they are the objective monoclonal antibody-producing cells. As the means of selecting the hybridomas that produce the antibody having the activity of blocking the IL-2 response from among the hybridomas that produce the antibody to the γ-chain molecule of the IL-2 receptor, any other process may be employed provided that it is a process of measuring the biological activity of human IL-2 using human cells, apart from the process using ILT-Mat cells. As the hybridoma clones thus obtained, for example, there are mentioned cells of GP-2 (FERM BP-4641), GP-4 (FERM BP-4640), TUGh4 (FERM BP-4642), TUGh5 (FERM BP-4643) and AG-14 (FEPM BP-4648).

For producing a large amount of the monoclonal antibody, it is possible that GP-2 cells (FERM BP-4641), GP-4 cells (FERM BP-4640), TUGh4 cells (FERM BP-4642), TUGh5 cells (FERM BP-4643) or AG14 cells (FERM BP-4648) are injected intraperitoneally to a tissue-adaptable animal, a thymoprival nude mouse or the like and grown therein and the antibody produced in the ascites of the animal is recovered and purified by salting-out, ion-exchanging chromatography, etc.

Next, a polypeptide comprising the V region of the monoclonal antibody capable of binding to the γ-chain of IL-2 receptor and therefore having an activity of blocking the IL-2 response may be produced, for example, in accordance with the process mentioned below.

First, total RNA is extracted from the hybridoma clone that produces the monoclonal antibody capable of binding to the γ-chain of IL-2 receptor and therefore having an activity of blocking the IL-2 response to obtain the gene (cDNA) coding for the V-region of the monoclonal antibody. We, the present inventors have assiduously designed the process for obtaining the objective cDNA as rapidly as possible and have obtained the cDNA coding for the V region of the antibody in accordance with the process mentioned below.

First, on the basis of the nucleotide sequences of the H-chain and the L-chain of a mouse IgG, of which the nucleotide sequence of the gene has already been reported, four kinds of DNA molecules (primer DNA's) each having a nucleotide sequence comprised of from 20 to 30 bases, which are highly common to the 5'-terminal and the 3'-terminal of the genes of the respective V-regions of the chains are designed. The 5'-side and the 3'-side hereinafter referred to are based on the sense strands of the H-chain V-region gene and the L-chain V-region gene. The 5'-side primer is a DNA molecule having the sequence on the sense strand, while the 3'-side primer is a DNA molecule having the complementary sequence at the 3'-side of the sense strand.

Next, an ATG sequence which is a translation-initiating codon is added to the 5'-side of the thus-designed L-chain V-region 5'-side primer, while a translation-terminating codon to the 3'-side of the 3'-side primer of the H-chain.

As a matter of course, the ATG sequence may be added to the 5'-side of the H-chain V-region 5'-side primer while the terminating codon to the 3'-side of the L-chain V-region 3'-side primer. As the terminating codon, usable is any of TAA, TAG and TGA. In the example of the present invention which will be mentioned hereinafter, TGA was used as the terminating codon.

A suitable restriction enzyme site into which an expression vector will be inserted is previously introduced into the 5'-terminals of the respective primer DNA's of the H-chain and the L-chain (the 5'-terminal as referred to herein indicates the 5'-terminal based on the primer molecule for the 3'-side primer). The thus-designed primer DNA is chemically synthesized, using a DNA synthesizer or the like.

Next, total RNA is extracted from the obtained hybridoma by a known method, a single-stranded cDNA is formed from a reverse transcriptase and the 3'-side primer DNA, and only the DNA fragments each coding for the V-region of the H-chain of the antibody and the V-region of the L-chain of the same are selectively amplified and collected by the polymerase chain reaction method (PCR method - see Science, Vol. 230, page 1350, 1985) using a Taq polymerase and using the 5'-side primer DNA and the 3'-side primer DNA.

In order that the gene coding for the H-chain V-region and the gene coding for the L-chain V-region are expressed in E. coli, etc. to produce a polypeptide comprising only the functional antibody V-region, the two genes are separately inserted into different two vectors so as to attain the intended expression therein, or alternatively, both the two genes are inserted into one and the same vector also so as to attain expression therein, and thereafter the polypeptide comprising the H-chain V-region and the polypeptide comprising the L-chain V region may be assembled together. However, it is known that the efficiency of the process is extremely poor. (See Science, Vol. 240, page 1038, 1988.)

Naturally, an antibody is composed of the combination of two molecules to be obtained by binding the H-chain and the L-chain together via SS-crosslinking by covalent bind. Namely, it has a structure of a dimer, in which the H-chains of the respective molecules together form SS bind. The position of the SS-crosslinking between the H-chain and the L-chain is on the constant region (hereinafter referred to as C-region), while the H-chain V-region and the L-chain V-region are bound to each other by non-covalent bind.

Therefore, where the polypeptide comprising the H-chain V-region and the polypeptide comprising the L-chain V-region are assembled into a polypeptide comprising only the antibody V-region, the association of the two molecules must rely upon only the non-covalent bind. For these reasons, it is considered that the efficiency in forming the functional molecule is poor. Recently, however, there has been developed a novel technique for linking a polypeptide comprising the H-chain V-region and a polypeptide comprising the L-chain V-region with a linker to give a single-stranded functional molecule. (See Science, Vol. 242, page 423, 1988.)

We, the present inventors, having applied the technique, have succeeded in the expression of a polypeptide comprising only the V-region of a functional, single-stranded anti-IL-2 receptor γ-chain antibody.

First, an expression vector is constructed, comprising a DNA containing a promoter region, a DNA containing a ribosome-binding region, a DNA containing a restriction enzyme site that has been introduced into the ATG sequence-added 5'-side primer, a DNA containing a restriction enzyme site that has been introduced into the 3'-side primer of the same chain, a DNA coding for a linker peptide having a suitable length that is to link the antibody L-chain V-region and the antibody H-chain V-region, a DNA containing a restriction enzyme site that has been introduced into the 5'-side primer having no ATG sequence, a DNA containing a restriction enzyme site that has been introduced into the 3'-side primer of the same chain, and last a DNA containing a terminator region, that have been arranged in this order from the upper stream.

Where the DNA coding for the H-chain V-region that has been amplified by PCR and the DNA coding for the L-chain V-region are both inserted, attention shall be paid in order that the translations of the DNA coding for the linker peptide and the DNA coding for the V-region are not mismatched.

The origin of the promoter to be employed in the present invention is not specifically defined. For instance, usable are trp promoter, tac promoter, trc promoter and lac promoter from E. coli; λPL promoter and λPR promoter from the λ phage; and also SV40 promoter, Moloney LTR promoter and CMV promoter from eucaryotic cells, etc. As the ribosome-binding region, for example, usable are the ribosome-binding regions of trpL, trpE and lacZ from E. coli, and the ribosome-binding region of CII protein from the λ phage. In addition, chemically synthesized DNA sequences may also be employed, as the case may be. If desired, the expression vector may have two or more ribosome-binding regions in order that a large amount of granules of the objective polypeptide are accumulated in the cells of E. coli.

The linker peptide for linking the polypeptide comprising the H-chain V-region and the polypeptide comprising the L-chain V-region may have any sequence, provided that the polypeptide comprising the antibody V-region, that is to be obtained by linking the two polypeptides with it, is functional. However, in order to minimize the harmful side effects of the functional polypeptide in administering it to living bodies, it is desired that the sequence of the linker peptide is as short as possible and has no peculiar structure.

As the terminator, for example, employable are trpA terminator, rrnB terminator and recA terminator from E. coli, etc. It is generally desired that the number of copies of the expression plasmid is as large as possible, and the pUC replication starting point is preferred over the pBR replication starting point.

The DNA coding for the polypeptide comprising the H-chain V region that has been amplified by PCR and the DNA coding for the polypeptide comprising the L-chain V-region are inserted into the thus-constructed expression vector to obtain a recombinant DNA. After the insertion, the recombinant DNA is used to transform a host by an ordinary method, whereby the gene on the recombinant DNA may be expressed in the host. As the host, employable is any of procaryotes and eucaryotes. As examples of procaryotes, mentioned are E. coli, Bacillus subtilis, etc. As examples of eucaryotes, mentioned are yeast, CHO cells, etc. As the host, preferred are procaryotes and more preferred is E. coli.

For transformation of the host with the recombinant DNA, any known method may be employed. For instance, for E. coli, the cells in the logarithmic growth stage are treated with 50 mM of calcium chloride in ice for about 30 minutes to thereby modify the structure of the cell wall of the cells of E. coli and subsequently the plasmid DNA is injected thereinto. After about 10 minutes, the cells are heat-treated at 30 • C to 42 • C for 2 minutes. Then, a medium is added thereto and the cells are incubated therein at 30 • C to 37 • C for about 60 minutes, whereby the recombinant DNA is introduced into the cells.

By incubating the cells that have been transformed with the recombinant DNA in the medium, the objective polypeptide comprising only the V-region of the monoclonal antibody to the β-chain of IL-2 receptor may be accumulated in the cells or in the medium. The medium may be any known one in which the cells may grow, and the conditions for the incubation may also be known ones. After the incubation, the objective polypeptide comprising only the V-region of the monoclonal antibody may be collected by a known method.

A polypeptide in which the C-region of the mouse monoclonal antibody capable of binding to the γ-chain of IL-2 receptor and therefore being able to block the IL-2 response has been changed into the C-region of a human antibody, and a polypeptide in which the constant region and the frame-work sequence of the V-region (hereinafter referred to as FR) of the mouse monoclonal antibody have been changed into the C-region and the FR, respectively, of a human antibody may be produced by the methods mentioned below.

First, a DNA having a length of from 20 to 30 bases corresponding to the nucleotide sequence of the 5'-terminal of the gene coding for the signal peptide of the H-chain or L-chain of the antibody is synthesized. Prior to the synthesis, a restriction enzyme site, at which the DNA is inserted into the downstream of the promoter region of the expression vector, is previously introduced into the 5'-terminal of the DNA to be synthesized. Subsequently, a DNA having a length of from 20 to 30 bases corresponding to the complementary sequence of the nucleotide sequence of the 3'-terminal of the gene coding for the V-region of the H-chain or L-chain of the antibody is synthesized. Also prior to the synthesis, a restriction enzyme site, at which the C-region cDNA of a human antibody is bound to the DNA, is previously arranged at the 3'-terminal of the DNA.

From the hybridoma that produces the mouse monoclonal antibody having an activity of blocking the IL-2 response, a total RNA is prepared by an ordinary method, from which produced is a single-stranded cDNA using a reverse transcriptase or the like. Afterwards, by PCR reaction using the thus-synthesized DNA as the primer, a DNA fragment coding for the signal peptide for the H-chain or L-chain and the V-region of the antibody is obtained.

In the same manner as above, the C-region gene of a human antibody may be obtained by preparing a total RNA from a human B-cell line or a human plasma cell line that produces an antibody and then forming a cDNA from the RNA using a reverse transcriptase or the like followed by subjecting the cDNA to PCR using a previously prepared DNA corresponding to the nucleotide sequence of the part of the 5'-terminal or the 3'-terminal of the C-region of the H-chain or the L-chain of the antibody. In the process, the same restriction enzyme site as the site that has been introduced into the 3'-side primer employed for obtaining the DNA fragment coding for the signal peptide of the H-chain or the L-chain and the V-region of the antibody is arranged at the outside of the 5'-terminal of the 5'-side primer; while a restriction enzyme site, at which the gene is inserted into the downstream of the promoter region of the expression vector, is arranged at the outside of the 5'-terminal of the 3'-side primer. Accordingly, the structure of the gene is so designed that the frames are not mismatched at the joint of the 3'-terminal of the V-region and the 5'-

terminal of the C-region. The class or subclass of the antibody to be prepared may be selected freely in accordance with the use and the object, by preparing a total RNA from the cells producing the antibody of the objective class or subclass followed by preparing the corresponding DNA coding for the C-region.

The thus-obtained DNA fragment coding for the signal peptide and the V-region of the mouse antibody and the DNA fragment coding for the C-region of the human antibody each are cut with the restriction enzyme corresponding to the restriction enzyme site that has been introduced into the respective fragments, and they are then mixed. Next, these DNA's are inserted into an expression vector expressible in animal cells, the expression vector having been cut with a restriction enzyme. The restriction enzyme to be used for cutting the expression vector is one which may yield the cut ends corresponding to those of the restriction enzyme site that is introduced into the DNA fragment coding for the signal peptide and the V-region of the mouse antibody and into the DNA fragment coding for the C-region of the human antibody from the PCR primer. Where the DNA fragment coding for the signal peptide and the V-region of the mouse antibody codes for those of the H-chain, it must be inserted into the expression vector along with the DNA fragment coding for the C-region of the human antibody H-chain. Similarly, where the DNA fragment coding for the signal peptide and the V-region of the mouse antibody codes for those of the L-chain, it must be inserted into the expression vector along with the DNA fragment coding for the C-region of the human antibody L-chain.

Finally, the expression vectors into which the DNA fragment coding for the signal peptide and the V-region of the mouse antibody and the DNA fragment coding for the C-region of the human antibody have been inserted each in the right direction are selected.

Both the thus-prepared expression vectors each containing a gene coding for the signal peptide and the V-region of the mouse antibody of the H-chain or L-chain and a gene coding for the C-region of the human antibody are transfected into animal cells by an ordinary method. As the host where the expression vectors are expressed, any cells may be employed.

After the transfection, the cells are cloned, and the binding activity of the antibody, which has been produced in the supernatant of the culture, to the γ-chain of the IL-2 receptor is measured. Accordingly, the objective polypeptide where the C-region of the mouse monoclonal antibody capable of binding to the γ-chain of the IL-2 receptor and therefore having an activity of blocking the IL-2 response has been changed to the C-region of the human antibody is obtained.

A polypeptide where the C-region and the FR of the V-region of the mouse monoclonal antibody has been changed into the C-region and the FR, respectively, of a human antibody may be produced by the process mentioned below. (See Medical Immunology, Vol. 22, No. 6, page 628. 1991) First, the amino acid sequence derived from the prepared DNA coding for the V-region of the mouse monoclonal antibody and the amino acid sequence of the V-region of a known human antibody are subjected to homology reference, by which the sequences with the highest homology are selected both for the H-chain and the L-chain. Next, the signal peptide and the FR part of the V-region are so designed that they may have the selected amino acid sequences of the human antibody, while the other V-region is so designed that it may have the amino acid sequence of the mouse monoclonal antibody. Then, DNA fragments coding for the peptide are designed and synthesized both for the H-chain and the L-chain. In this process, the DNA fragments are divided into segments each having a length of approximately from 30 to 40 bases while approximately from 5 to 7 bases are made to overlap at the joints of the segments, and these are synthesized to have a complementary sequence to the original sequence on alternate segments. At the outside of the 5'-terminal of the signal peptide and the 3'-terminal of the V-region, arranged are an expression vector and a restriction enzyme site at which the DNA fragment is bound to the human C-region. These synthetic DNA fragments are mixed and subjected to PCR, and the recovered DNA fragments coding for the signal peptide and the V-region of the H-chain and the L-chain of the antibody are cut with the restriction enzyme corresponding to the introduced restriction enzyme site. Using the similar restriction enzyme, the DNA fragments coding for the signal peptide and the V-region of the H-chain and the L-chain are cut and separated from the previously prepared vectors that express the polypeptides where the C-region of the H-chain and the L-chain of the mouse monoclonal antibody has been changed into the C-region of the human antibody, and they are substituted for the previously prepared DNA fragment coding for the signal peptide and the V-region. The thus-constructed recombinant DNA are transfected into animal cells by the same process as that mentioned above, and the cells that express the objective polypeptide are selected also by the same process as above. Accordingly, the objective polypeptide where the C-region and the FR of the V-region of the mouse monoclonal antibody capable of binding to the γ-chain of the IL-2 receptor and therefore having an activity of blocking the IL-2 response have been changed into the V-region and the FR, respectively, of the human antibody is obtained.

The polypeptide of the present invention specifically binds to the γ-chain of the IL-2 receptor and therefore blocks the response to IL-2, and it is effective in preventing the rejection after transplantation and in curing autoimmune diseases, etc.

The monoclonal antibody of the present invention is not defined to only the monoclonal antibody to be produced by the hybridoma clone obtained herein but includes any other monoclonal antibodies capable of binding to the γ-chain of the IL-2 receptor and therefore being able to block the IL-2 response. In addition, a chimera antibody to be prepared by changing the C-region of the monoclonal antibody of the present invention into a human C-region by a known method and an antibody to be prepared by changing the FR of the V-region of the same into a human FR are also within the scope of the monoclonal antibody of the present invention, provided that they bind to the γ-chain of the IL-2 receptor and therefore have the activity of blocking the IL-2 response.

The structure of the polypeptide of the present invention has, for example, the sequence of Sequence No. (I) or No. (II) shown in Table of Sequences, which, however, is not limited thereto. The present invention includes any other polypeptides capable of binding to the γ-chain of IL-2 receptor and therefore being able to block the IL-2 response. For instance, it includes (1) a polypeptide having a polypeptide structure of Sequence No. (I) or No. (II) in which one or more amino acid residues have been substituted by other amino acid residue(s), (2) a polypeptide having a continuous amino acid sequence of Sequence No. (I) or No. (II) in which one or more amino acid residues have been deleted from the N-terminal and/or the C-terminal, (3) a polypeptide having a structure of Sequence No. (I) or No. (II) in which one or more amino acid residues have been added to the N-terminal and/or the C-terminal, (4) a polypeptide having a structure of Sequence No. (I) or No. (II) in which one or more amino acid residues have been acetylated, amidated or modified with polyethylene glycol(s), etc., provided that they bind to the γ-chain of IL-2 receptor and therefore have an activity of blocking the IL-2 response.

In particular, Met at the N-terminal of the polypeptide having a structure of Sequence No. (I) or No. (II) in Table of Sequences is often cut during the process of expressing it using microorganisms or during the process of purifying it, with the result that its N-terminal is changed into Asp. The resulting polypeptide also has the above-mentioned function or, that is, also binds to the γ-chain of IL-2 receptor and therefore has an activity of blocking the IL-2 response. The polypeptide of the present invention that has been prepared in the form having Met at its N-terminal may be treated with an enzyme such as aminopeptidase or the like to remove Met from its N-terminal, and the resulting polypeptide also has the above-mentioned function.

If desired, a toxin may be added to the monoclonal antibody or polypeptide of the present invention.

The immunosuppressant of the present invention may contain the above-mentioned monoclonal antibody or polypeptide in an amount of from 0.1% by weight to 100% by weight, preferably from 0.5% by weight to 70% by weight. Therefore, the monoclonal antibody or polypeptide of the present invention may be administered directly as it is or, alternatively, in the form of a medicinal preparation to be prepared by blending it and an ordinary pharmaceutical carrier. As the pharmaceutical carrier, usable are substances which are ordinarily used in the pharmaceutical field and which do not react with the monoclonal antibody and the polypeptide of the present invention. An injection may be prepared by dissolving the monoclonal antibody or polypeptide of the present invention in water. If desired, it may be dissolved in a physiological saline solution or glucose solution. The injection may optionally contain a buffer, a preservative, a stabilizer and a vehicle. These medicinal preparations of the present invention may contain other therapeutically valuable components.

To administer the immunosuppressant of the present invention, any of peroral, injectable and per-rectal routes may be employed, but preferably it is administered by injection. The dose of the immunosuppressant varies, depending on the administration route, the condition and the age of the patient, etc. In general, the medicine may be administered once to three times a day each in an amount of from 0.001 to 1000 mg, preferably from 0.01 to 10 mg.

The present invention will be explained in more detail by means of the following examples, which, however, are not intended to restrict the scope of the present invention.

EXAMPLE 1:

Preparation of recombinant human IL-2 receptor γ-chain polypeptide comprising only extracellular region:

To prepare an IL-2 receptor γ-chain cDNA having a stop codon at its 3'-terminal in the extracellular region, an oligomer 5'-GGACATATGCTGAACACGACAATTCTG-3' (Sequence No. 3) having an NdeI site in its inside and an oligomer 5'-GAAAAGCTTCTATTATGAAGTATTGCTCC-3' (Sequence No. 4) having an HindIII site in its inside were integrated, using a DNA synthesizer 380A Model. The both oligomers, being

used as the primers, were subjected to PCR (having 20 cycles each comprising denaturation at 94 • C, annealing at 55 • C and synthesis at 72 • C) with Taq polymerase, using a plasmid containing the cDNA of IL-2 receptor γ-chain molecule (E. coli that had been transformed with this plasmid has been deposited with the National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology as the deposition code number of FERM BP-4200), as the template, and using a thermal cycler.

The amplified band with about 0.7 kb was recovered, cut with NdeI and HindIII (made by TAKARA SHUZO CO., LTD.) and ligated with the large fragment of plasmid pFv(TU27)-DE (E. coli that had been transformed with this plasmid has been deposited in the National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology as the deposition code number of FERM BP-3973) that has been obtained by cutting the plasmid with NdeI and HindIII followed by collecting the large fragment, to construct pIL-2RGS. (See Fig. 1.)

Cells of E. coli HB101 that had been transformed with pIL-2RGS were incubated in an M9-Casamino acid medium, whereby the protein was expressed in the cells of E. coli as granules. The cells were ultrasonically disrupted and subjected to centrifugation of 3,000 x g to isolate the granules. The granules were dissolved in 6 M guanidine hydrochloride and then the protein was refolded by stirring the resulting solution overnight at room temperature in the presence of 3.5 M, as the final concentration, of guanidine hydrochloride, 30 μM, as the same, of reduced glutathione and 3 μM, as the same, of oxidized glutathione and under the condition that the protein concentration was 50 μg/ml. This was subjected to dialysis against 10 mM phosphate buffer containing 150 mM NaCl (pH 7.5) (hereinafter referred to as PBS), to prepare a recombinant human IL-2 receptor γ-chain polypeptide comprising only the soluble extracellular region.

EXAMPLE 2

Preparation of hybridomas:

Female BALB/c mice of from 6 to 8 weeks age were immunized with the recombinant human IL-2 receptor γ-chain polypeptide comprising only the extracellular region, by subcutaneously injecting 100 μg/mouse of the polypeptide to the mice along with a Freund's complete adjuvant (made by Bacto Co.). The immunization was repeated additionally two times at intervals of 3 weeks by the same operation, and the blood was collected from the supraorbital vein of each of the immunized mice. The blood was examined according to the method which will be mentioned hereinafter to determine the antibody value by measuring the bound amount of the recombinant human IL-2 receptor γ-chain polypeptide comprising only the extracellular region in the blood. The mice having a high antibody value were finally immunized by the same operation. Three days after the final immunization, the spleen was taken out from each mice. The cells of the spleen were fused with mouse myeloma cells (SP2/0-Ag14) in the presence of 50% polyethylene glycol #4000 (made by Nacalaitesque Co.), by mixing them at a ratio of 10:1 in terms of the number of the cells.

The fused cells were suspended in RPMI1640 medium (made by Gibco Co.) containing 10% fetal calf serum (made by Gibco Co.) in an amount of 5 x 106 cells/ml, and the resulting suspension was put into the wells of a 96-well flat-bottomed plate (made by Corning Co.) containing 5 x 10^5 mouse thymocytes in each well, in an amount of 100 μl/well. After 1, 2, 3 and 6 days, a half of the medium was exchanged for a medium containing hypoxanthine, aminopterin and thymidine (HAT medium), and thereafter the same operation was repeated at intervals of 3 days. About 2 weeks after the cell fusion, the amount of the antibody in the supernatant of the culture in each well where the fused cells (hybridomas) was growing, which had bound to the Lβ cells, as well as the amount of the same which had bound to the Lβ cells was measured, and the hybridomas that bound to only the Lγ cells were cloned by a limiting dilution-culture method.

In addition, in the same manner as mentioned above, the amounts of the hybridoma clones that had bound to the cells in the supernatant of the culture were measured, and anti-IL-2 receptor γ-chain antibody-producing hybridomas were obtained. The supernatant of the culture of the thus-obtained anti-IL-2 receptor γ-chain antibody-producing hybridomas was examined with respect to their potency of inhibiting the biological activity of IL-2 in accordance with the method mentioned below. Precisely, a suspension of ILT-Mat cells that had been suspended in RPMI1640 medium containing 10% fetal calf serum (FCS) in a concentration of 2 x 10^5 cells/ml was put into the wells of a 96-well flat-bottomed micro-plate in an amount of 100 μl/well, 50 μl/well of the supernatant of the culture of the sample to be tested was added to the wells, and 50 μl/well of a human recombinant IL-2 solution that had been prepared by dissolving 200 U/ml of the human recombinant IL-2 in RPMI1640 medium containing 10% FCS was added thereto. The cells were then incubated at 37 • C for 48 hours in the presence of 5% CO2. In the last four hours, the incubation

was continued while 1 μCi of 3H-thymidine (made by DuPont Co.) was added to the wells, whereupon the intake of the radiation-active amount that had been taken into the cells was measured with a scintillation counter (made by Packard Co.). From the measured amount, the inhibition of the biological activity of IL-2 by the supernatant of the culture was determined. Accordingly, the hybridomas that produce the antibody to IL-2 receptor γ-chain molecules were prepared. The thus-obtained hybridomas are GP-2 (FERM BP-4641), GP-4 (FERM BP-4640), TUGh4 (FERM BP-4642), TUGh5 (FERM BP-4643) and AG14 (FERM BP-4648).

The antibody to be produced by hybridoma GP-2 is referred to as antibody GP-2, and that to be produced by hybridoma GP-4 as antibody GP-4. The antibody to be produced by hybridoma TUGh4 is referred to as antibody TUGh4, and that to be produced by hybridoma TUGh5 as antibody TUGh5. The antibody to be produced by hybridoma AG14 is referred to as antibody AG14.

EXAMPLE 3

Preparation of cDNA coding for only V-region of antibody:

5 x 106 hybridomas GP-2 or GP-4 were washed with PBS and suspended in an RNA-extracting buffer containing guanidine thiocyanate, N-lauryl sarcosine and EDTA (made by Pharmacia Co.). The hybridoma suspension was layered over caesium chloride solution (ρ = 1.51 g/ml, made by Pharmacia Co.) that had been put in a tube, which was subjected to centrifugation at 125,000 x g for 16 hours. The volume of the hybridoma suspension was the same as the volume of the caesium chloride solution. After the supernatant was removed by suction, 10 mM tris-hydrochloride solution (pH 7.5) containing 1 mM EDTA was added to the pellet so that the pellet was suspended in the buffer. The resulting suspension was put in a new tube and incubated therein at 65 • C for 5 minutes. 1/10 volume of 2 M potassium acetate (pH 5.0) (made by Pharmacia Co.) and three times volume of ethanol (made by Nacalaitesque Co.) were added thereto and allowed to stand at -20 • C overnight. This was subjected to centrifugation at 5,000 x g for 20 minutes, the supernatant was removed, and the resulting pellet was washed with 80% ethanol and then dried.

The pellet was dissolved in 10 mM tris-hydrochloride buffer (pH 7.5) containing 1 mM EDTA to obtain a total RNA fraction solution.

Next, to the total RNA fraction solution, added were a solution of the 3'-side primer of the V-region of the H-chain of the antibody (1 μm as the final concentration), a solution of the 3'-side primer of the V region of the L-chain of the same (1 μm as the final concentration), a deoxy NTP mixture, a buffer for synthesis of cDNA (made by Amersham Co.), an RNAase inhibitor (made by TAKARA SHUZO CO., LTD.) and a reverse transcriptase (made by TAKARA SHUZO CO., LTD.), and reacted at 42 • C for one hour to produce a cDNA.

To the thus-obtained cDNA, added were a 5'-side primer and a 3'-side primer that are used for amplifying the cDNA coding for only the antibody H-chain V-region (each 1 μM as the final concentration), a 5'-side primer and a 3'-side primer that are used for amplifying the cDNA coding for only the antibody L-chain V-region (each 1 μM as the final concentration), a deoxy NTP mixture, a buffer for PCR (made by Perkin-Elmer Co.) and Taq polymerase (made by TAKARA SHUZO CO., LTD.), and subjected to PCR using a thermal cycler (made by Perkin-Elmer Co.). One cycle of the reaction comprised 30 seconds of denaturation (at 94 • C), 30 seconds of annealing (at 55 • C) and one minute of primer extension (at 72 • C), and 30 cycles were repeated. At every cycle, the time for the primer extension was prolonged by 15 seconds.

After the reaction, the reaction mixture was subjected to agarose gel electrophoresis, using 40 mM tris-acetic acid buffer (pH 8.0) containing 1 mM EDTA, and the corresponding cDNA fragments were cut out, extracted and purified with a geneclean kit (made by Bio 101 Co.). The sequences of the primers used for the synthesis of cDNA and for the PCR are shown in Fig. 2 (Sequence Nos. 5 to 8).

EXAMPLE 4

Construction of an Expression Vector:

First, as shown in Fig. 4, the large DNA fragment that had been obtained by cutting pT13SNco (E. coli AJ-12447 containing this plasmid has been deposited with the National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology as FERM BP-4200) [described in J. Biochem., Vol 104, page 30, 1988] with restriction enzymes ClaI and BamHI (both made by TAKARA SHUZO CO., LTD.) and the DNA fragment having the sequence shown in Fig. 3 (linker, Sequence Nos. 9 and 10) were ligated together, using a T4 DNA ligase (made by TAKARA SHUZO CO., LTD.). The DNA fragment having the sequence shown in Fig. 3 was produced, using a DNA synthesizer.

Next, the plasmid that had been obtained by ligating the large ClaI-BamHI fragment derived from plasmid pT12SNco and the synthetic DNA fragment was cut with restriction enzymes EcoRI and PvuII (both made by TAKARA SHUZO CO., LTD.) to obtain a small DNA fragment (hereinafter referred to as fragment A, for convenience' sake).

On the other hand, pUC18 (see Methods in Enzymology, Vol. 101, page 20, 1983) was cut with a restriction enzyme HindIII, the cut ends were made blunt using T4 DNA polymerase (made by TAKARA SHUZO CO., LTD.), and this was self-ligated with T4 ligase to remove the HindIII site therefrom The pUC18 from which the HindIII site had been removed was cut with restriction enzyme NdeI, the cut ends were made blunt using T4 DNA polymerase (made by TAKARA SHUZO CO., LTD.), and this was self-ligated with T4 ligase to remove the NdeI site therefrom.

The large DNA fragment that had been obtained by cutting pUC18, from which both the HindIII site and the NdeI site had been removed, with EcoRI and HincII (made by TAKARA SHUZO CO., LTD.) and the fragment A were ligated with T4 ligase to obtain plasmid pFv-DE having a pUC replication-starting point.

EXAMPLE 5

Insertion of an antibody V-region cDNA into pFv-DE, and preparation of microorganisms producing an antibody comprising only the V-region:

First, as shown in Fig. 5, the pFv-DE was cut with restriction enzymes NdeI and SalI (made by TAKARA SHUZO CO., LTD.) to obtain a large DNA fragment. The large DNA fragment was ligated with the fragment that had been obtained by cutting the L-chain V-region cDNA of GP-2 obtained in Example 3 with NdeI and SalI, using a T4 ligase. Similarly, the large fragment was ligated with the fragment that had been obtained by cutting the L-chain V-region cDNA of GP-4 also obtained in Example 3 with NdeI and SalI, using a T4 ligase.

The thus-obtained two plasmids each were cut with restriction enzymes XhoI and HindIII (made by TAKARA SHUZO CO., LTD.) to obtain large fragments.

Of the large DNA fragments, one containing the L-chain V-region cDNA of GP-2 was ligated with the fraction obtained by cutting the H-chain V-region cDNA of GP-2 that had been obtained in Example 3, with XhoI and HindIII, using a T4 ligase. Similarly, the other large DNA fragment containing the L-chain V-region cDNA of GP-4 was ligated with the fraction obtained by cutting the H-chain V-region cDNA of GP-4 that had been obtained in Example 3, with XhoI and HindIII, using a T4 ligase.

Accordingly, two plasmids expressing an antibody comprising only the V-region, pFv(GP-2)-DE and pFv(GP-4)-DE were obtained.

Subsequently, E. coli HB101 was transformed with each of the plasmids to obtain E. coli pFv(GP-2)-DE/HB101 (AJ-12844, FERM BP-4636) and E. coli pFv(GP-4)-DE/HB101 (AJ-12845, FERM BP-4637), which produce an antibody comprising only the V-region.

EXAMPLE 6

Collection of the product from microorganisms producing an antibody comprising only the V-region:

The thus-obtained transformants, E. coli pFv(GP-2)-DE/HB101 (AJ-12844, FERM BP-4636) and E. coli pFv(GP-4)-DE/HB101 (AJ-12845, FERM BP-4637) each were grown in 5 ml of 2xYT [1.6% trypton, 1% yeast extract (both made by Bacto Co.), 0.5% NaOH, pH 7.0] containing 50 μg/ml of ampicillin, at 37°C overnight. Next, 5 ml of each of the culture suspensions was seeded in 100 ml of M9-Casamino medium (0.6% $Na_2HPO_4.12H_2O$, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.05% $MgSO_4.7H_2O$), 0.00147% $CaCl_2$, 0.2% glucose, 0.2% Casamino acid, 0.02% L-leucine, 0.02% L-proline, 0.0002% thiamine hydrochloride, 100 μg/ml ampicillin, pH 6.9) and incubated therein at 37°C for 3 hours. Afterwards, 3-indole-acrylic acid (IAA) was added thereto to have a final concentration of 25 μg/ml therein, and the mixture was further incubated at 37°C for 20 hours by induction cultivation. A part of the suspension of the grown cells was observed with a phase contrast microscope with about 1500 powers, which revealed the formation of granules in the cells of E. coli.

The suspension of the cells that had been incubated in the manner as mentioned above were subjected to centrifugation to collect the cells, which were then suspended in 50 ml of 30 mM Tris-HCl buffer (pH 7.5) containing 30 mM NaCl. 12.5 ml of an aqueous solution of 0.5 M EDTA (pH 8.0) containing 1 mg/ml of lysozyme were added to the suspension, which was then stirred and kept in ice for one hour. Next, the cells were ultrasonically disrupted and subjected to centrifugation at 6000 rpm for 5 minutes to recover the

granules therefrom. The granules were dissolved in 6 M guanidine hydrochloride solution, and the resulting solution was adjusted so that the concentration of the objective polypeptide became 100 μg/ml and that of guanidine hydrochloride 3.5 M. Afterwards, oxidized glutathione and reduced glutathione were added thereto in such a way that the final concentration of the former was 3 μM and that of the latter 30 μM. Subsequently, the pH of the solution was adjusted at 8.0, and the solution was allowed to stand at room temperature for 10 to 16 hours. Finally, the solution was subjected to dialysis against PBS to obtain the objective antibody comprising only the V-region. Both the two transformants were processed in accordance with the above-mentioned process to obtain two antibodies, one being referred to as polypeptide Fv(GP-2) and the other as polypeptide Fv(GP-4).

The molecular weights of the two polypeptides were determined by SDS polyacrylamide gel electrophoresis. The thus-determined molecular weights almost corresponded to those calculated on the basis of the respective amino acid sequences that had been presumed in accordance with Example 7 mentioned below.

Using a protein sequencer, the amino acid sequence of the N-terminal side of each of these polypeptides was sequenced, with the result that the two polypeptides each were identified to have the same amino acid sequence as that presumed in the following Example 7.

EXAMPLE 7

Determination of nucleotide sequence:

The thus-constructed plasmids pFv(GP-2)-DE and pFv(GP-4)-DE each expressing a polypeptide comprising only the V-region were purified by an alkali SDS method. Using a sequence kit, 7-DEAZA Model (made by TAKARA SHUZO CO., LTD.) and using a commercial sequencing primer M4 or RV (made by TAKARA SHUZO CO., LTD.), the nucleotide sequences of these plasmids were determined. On the basis of the thus-obtained nucleotide sequences, their amino acid sequences were derived.

Sequence No. (I) indicates the nucleotide sequence of the DNA coding for the polypeptide Fv(GP-2) along with the amino acid sequence thereof to be derived from the nucleotide sequence. Sequence No. (II) indicates the nucleotide sequence of the DNA coding for the polypeptide Fv(GP-4) along with the amino acid sequence thereof to be derived from the nucleotide sequence.

As is noted from Sequence No. (I), Fv(GP-2) is a polypeptide comprising 244 amino acids, having Met at its N-terminal and Ser at its C-terminal. Fv(GP-4) is a polypeptide comprising of 243 amino acids, having Met at its N-terminal and Ser at its C-terminal.

The L-chain V-region of the antibody produced by GP-2 corresponds to the sequence composed of from the 2nd to 109th amino acids in the amino acid sequence of Sequence No. (I), while the H-chain V-region thereof corresponds to the sequence composed of from 124th to 244th amino acids in the same. The L-chain V-region of the antibody to be produced by GP-4 corresponds to the sequence composed of from the 2nd to 108th amino acids in the amino acid sequence of Sequence No. (II), while the H-chain V-region thereof corresponds to the sequence composed of from 123th to 243th amino acids in the same.

EXAMPLE 8

Determination of the activities of antibody GP-2, antibody GP-4, polypeptide Fv(GP-2) and polypeptide Fv-(GP-4):

ILT-Mat cells were suspended in RPMI1640 medium containing 10% FCS in a concentration of 2 x 105 cells/ml. The suspension was put into the wells of a 96-well, flat-bottomed micro-plate The amount of the ILT-Mat cell suspension that had been put into each well was 100 μl/well. The sample solution to be tested was added to the wells. The amount of the sample solution that had been added to each well was 50 μl, and the amount of the antibody or polypeptide contained in the solution was 40 μg/ml.

After having incubated at 37 • C for 30 minutes, 50 μl/well of a solution of human recombinant IL-2 that had been prepared by adding a varying amount of human recombinant IL-2 to RPMI1640 medium containing 10% FCS was added to the wells and the cells were incubated for further 48 hours at 37 • C in the presence of 5% CO2. In the last four hours, the incubation was continued while 1 μCi of 3H-thymidine (made by DuPont Co.) was added to the wells.

The intake of the radiation-active amount that had been taken into the cells was measured with a scintillation counter (made by Packard Co.). From the measured amount, determined was the response-blocking potency of each of the antibody GP-2, the antibody GP-4, the polypeptide Fv(GP-2) and the

polypeptide Fv(GP-4).

The results obtained are shown in Fig. 6, from which it has become clarified that the antibody GP-2, the antibody GP-4, the polypeptide Fv(GP-2) and the polypeptide Fv(GP-4) all have an activity of blocking the IL-2 response in the ILT-Mat cells.

EXAMPLE 9

Determination of IL-2 response-blocking activity of the monoclonal antibody GP-2 in the presence of anti-IL-2 receptor α-chain antibody and/or anti-IL-2 receptor β-chain antibody:

ILT-Mat cells were suspended in RPMI1640 medium containing 10% FCS in a concentration of 4 x 105 cells/ml. The suspension was put into the wells of a 96-well, flat-bottomed micro-plate. The amount of the ILT-Mat cell suspension that had been put into each well was 100 µl/well. The sample solution to be tested was added to the wells. The amount of the sample solution that had been added to each well was 50 µl, and the amount of each of antibody GP-2, anti-IL-2 receptor α-chain antibody and anti-IL-2 receptor β-chain antibody contained in the sample solution was 40 µg/ml.

After having incubated at 37 • C for 30 minutes, 50 µl/well of a solution of human recombinant IL-2 that had been prepared by adding a varying amount of human recombinant IL-2 to RPMI1640 medium containing 10% FCS was added to the wells and the cells were incubated for further 48 hours at 37 • C in the presence of 5% CO2. In the last four hours, the incubation was continued while 1 µCi of 3H-thymidine (made by DuPont Co.) was added to the wells.

The intake of the radiation-active amount that had been taken into the cells was measured with a scintillation counter (made by Packard Co.). From the measured amount, determined was the IL-2 response-blocking potency of the antibody GP-2 in the presence of anti-IL-2 receptor α-chain antibody and/or anti-IL-2 receptor β-chain antibody.

The results obtained are shown in Fig. 7, from which it has become clarified that the antibody GP-2 blocks more effectively the IL-2 response in the ILT-Mat cells in the presence of anti-IL-2 receptor α-chain antibody and/or anti-IL-2 receptor β-chain antibody.

EXAMPLE 10

Determination of the activities of the antibody TUGh4, the antibody TUGh5 and the antibody AG14:

ILT-Mat cells were suspended in RPMI1640 medium containing 10% FCS in a concentration of 2 x 10^5 cells/ml. The suspension was put into the wells of a 96-well, flat-bottomed micro-plate. The amount of the ILT-Mat cell suspension that had been put into each well was 100 µl/well. The sample solution to be tested was added to the wells. The amount of the sample solution that had been added to each well was 50 µl, and the amount of the antibody or polypeptide contained in the sample solution was 40 µg/ml.

After having incubated at 37 • C for 30 minutes, 50 µl/well of a solution of human recombinant IL-2 that had been prepared by adding a varying amount of human recombinant IL-2 to RPMI1640 medium containing 10% FCS was added to the wells and the cells were incubated for further 48 hours at 37 • C in the presence of 5% CO2. In the last four hours, the incubation was continued while 1 µCi of ^{3}H-thymidine (made by DuPont Co.) was added to the wells.

The intake of the radiation-active amount that had been taken into the cells was measured with a scintillation counter (made by Packard Co.). From the measured amount, determined was the IL-2 response-blocking potency of each of the antibody TUGh4, the antibody TUGh5 and the antibody AG14.

From the results obtained, it has become clarified that the antibodiy TUGh4, the antibody TUGh5 and the antibody AG14 have an activity of blocking the IL-2 response in the ILT-Mat cells.

REFERENTIAL EXAMPLE

Determination of the activity of the antibody TUGm2:

Using a monoclonal antibody, TUGm2 (this is anti-mouse IL-2 receptor γ-chain antibody that was prepared by immunizing rats in accordance with the process of Example 2) and/or a monoclonal antibody, anti-mouse IL-2 receptor β-chain antibody (TM-β1; made by Pharmingen Co.), the IL-2 response-blocking potency of these antibodies, if any, in CTLL-2 cells was determined in accordance with the process mentioned below.

Precisely, CTLL-2 cells were suspended in RPMI1640 medium containing 10% FCS in a concentration of 1 x 10^4 cells/ml. The suspension was put into the wells of a 96-well, flat-bottomed micro-plate. The amount of the CTLL-2 cell suspension that had been put into each well was 50 μl/well. The sample solution to be tested was added to the wells. The amount of the sample solution that had been added to each well was 50 μl, and the amount of each antibody contained in the sample solution was 40 μg/ml.

After having incubated at 37 • C for 30 minutes, 50 μl/well of a solution of human recombinant IL-2 that had been prepared by adding a varying amount of human recombinant IL-2 to RPMI1640 medium containing 10% FCS was added to the wells. In addition, RPMI1640 medium containing 10% FCS was further added to the wells, whereupon the final volume of the liquid in each well became 200 μl.

The cells were incubated for further 48 hours at 37 • C in the presence of 5% CO2. In the last four hours, the incubation was continued while 1 μCi of ^{3}H-thymidine (made by DuPont Co.) was added to the wells.

The intake of the radiation-active amount that had been taken into the cells was measured with a scintillation counter (made by Packard Co.). From the measured amount, determined was the IL-2 response-blocking potency of the monoclonal antibody TUGm2 and/or the anti-mouse IL-2 receptor β-chain antibody.

The results obtained are shown in Fig. 8, from which it has become clarified that the monoclonal antibody TUGm2 blocks the IL-2 response in the CTLL-2 cells in the presence of anti-mouse IL-2 receptor β-chain antibody.

As has been explained in detail hereinabove, the novel polypeptide of the present invention specifically binds to the γ-chain of human interleukin-2 receptor to selectively inhibit the binding of the γ-chain of human interleukin-2 receptor to the β-chain of the same, therefore having an activity of blocking the human interleukin-2 response. The novel polypeptide is a valuable substance which is usable, independently or along with substances capable of inhibiting the binding of interleukin-2 to interleukin-2 receptor, as a medicine effective in preventing the rejection after transplantation and also in curing inflammatory diseases such as allergic diseases and autoimmune diseases, the possibility that interleukin-2 will participate in the rejection and also in such inflammatory diseases having been suggested.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

SEQUENCE LISTING

GENERAL INFORMATION:

APPLICANT:
NAME: Ajinomoto Co., Inc.
STREET: 15-1, Kyobashi 1-chome
CITY: Chuo-ku, Tokyo
COUNTRY: Japan
POSTAL CODE: 104
TELEPHONE: (03)5250-8111
TELEFAX: (03)5250-8347
TELEX: J22690

TITLE OF INVENTION: Immunosuppressant

NUMER OF SEQUENCES: 12

COMPUTER READABLE FORM:
MEDIUM TYPE: Diskette
COMPUTER: IBM PC compatible
OPERATING SYSTEM: MS-DOS

INFORMATION FOR SEQ ID NO:1:

SEQUENCE CHARACTERISTICS:
LENGTH: 244 amino acids
TYPE: amino acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: no
ORIGINAL SOURCE: mouse
SEQUENCE DESCRIPTION:

```
Met Asp Ile Leu Leu Thr Gln Ser Pro Ser Ile Met Ser Ala Ser Leu
 1               5                  10                  15
Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser
            20                  25                  30
Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu
        35                  40                  45
Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe
    50                  55                  60
Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met
65                  70                  75                  80
Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser
                85                  90                  95
Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Val Asp Lys
            100                 105                 110
Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln Val Lys Leu Glu
        115                 120                 125
Glu Ser Gly Ser Glu Leu Val Arg Pro Gly Ala Ser Val Lys Leu Ser
    130                 135                 140
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Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met His Trp Val
145                 150                 155                 160
Lys Gln Arg His Gly Gln Gly Leu Glu Trp Ile Gly Asn Ile Tyr Pro
                    165                 170                 175
Gly Ser Gly Ser Thr Asn Tyr Asp Glu Lys Phe Lys Ser Lys Ala Thr
            180                 185                 190
Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr Met His Leu Ser Ser
        195                 200                 205
Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Thr Arg Ser Ser Arg
    210                 215                 220
Asn Trp Val Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
225                 230                 235                 240
Thr Val Ser Ser
```

INFORMATION FOR SEQ ID NO:2:

SEQUENCE CHARACTERISTICS:
LENGTH: 243 amino acids
TYPE: amino acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: no
ORIGINAL SOURCE: mouse
SEQUENCE DESCRIPTION:

```
Met Asp Ile Leu Leu Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val
  1                 5                  10                  15
Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn
             20                  25                  30
Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu
         35                  40                  45
Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser
     50                  55                  60
Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln
 65                  70                  75                  80
Pro Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro
                 85                  90                  95
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Val Asp Lys Ser
            100                 105                 110
Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln Val Lys Leu Glu Glu
        115                 120                 125
Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
    130                 135                 140
Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Tyr Met His Trp Val Lys
145                 150                 155                 160
Gln Ser His Val Lys Ser Leu Glu Trp Ile Gly Arg Ile Asn Pro Tyr
                165                 170                 175
Asn Gly Ala Thr Ser Tyr Asn Gln Asn Phe Lys Asp Lys Ala Ser Leu
            180                 185                 190
Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu His Ser Leu
        195                 200                 205
Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Tyr
    210                 215                 220
```

Gly Ser Ser Tyr Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
225 230 235 240
Val Ser Ala

INFORMATION FOR SEQ ID NO:3

SEQUENCE CHARACTERISTICS:
LENGTH: 732 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: no
ORIGINAL SOURCE: mouse
SEQUENCE DESCRIPTION:

ATGGACATCC TGCTGACCCA GTCTCCATCA ATCATGTCTG CATCTCTAGG GGAACGGGTC 60
ACCATGACCT GCACTGCCAG CTCAAGTGTA AGTTCCAGTT ACTTGCACTG GTACCAGCAG 120
AAGCCAGGAT CCTCCCCCAA ACTCTGGATT TATAGCACAT CCAACCTGGC TTCTGGAGTC 180
CCAGCTCGCT TCAGTGGCAG TGGGTCTGGG ACCTCTTACT CTCTCACAAT CAGCAGCATG 240
GAGGCTGAAG ATGCTGCCAC TTATTACTGC CACCAGTATC ATCGTTCCCC GCTCACGTTC 300
GGTGCTGGGA CCAAGCTGGA GCTCAAAGTC GACAAATCCT CAGGATCTGG CTCCGAATCC 360
AAAAGCACGC AGGTCAAACT CGAGGAGTCT GGATCTGAGC TGGTGAGGCC TGGAGCTTCA 420
GTGAAGCTGT CCTGCAAGGC TTCTGGCTAC ACATTCACCA GCTACTGGAT GCACTGGGTG 480
AAGCAGAGGC ATGGACAAGG CCTTGAGTGG ATTGGAAATA TTTATCCTGG TAGTGGTAGT 540
ACTAACTACG ATGAGAAGTT CAAGAGCAAG GCCACACTGA CTGTAGACAC ATCCTCCAGC 600
ACAGCCTACA TGCACCTCAG CAGCCTGACA TCTGAGGACT CTGCGGTCTA TTACTGTACA 660
AGAAGCAGCC GGAACTGGGT CTACTATGCT ATGGACTACT GGGGTCAAGG AACCTCAGTC 720
ACCGTCTCCT CA 732

INFORMATION FOR SEQ ID NO:4

SEQUENCE CHARACTERISTICS:
LENGTH: 729 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: no
ORIGINAL SOURCE: mouse
SEQUENCE DESCRIPTION:

ATGGATATTC TGCTGACACA GTCTCCAGCC TCCCTATCTG CATCTGTGGG AGAAACTGTC 60
ACCATCACAT GTCGAGCAAG TGGGAATATT CACAATTATT TAGCATGGTA TCAGCAGAAA 120
CAGGGAAAAT CTCCTCAGCT CCTGGTCTAT AATGCAAAAA CCTTAGCAGA TGGTGTGCCA 180
TCAAGGTTCA GTGGCAGTGG ATCAGGAACA CAATATTCTC TCAAGATCAA CAGCCTGCAG 240
CCTGAAGATT TTGGGAGTTA TTACTGTCAA CATTTTTGGA GTACTCCGTG GACGTTCGGT 300
GGAGGGACCA AGCTGGAGCT CAAAGTCGAG AAATCCTCAG GATCTGGCTC CGAATCCAAA 360
AGCACGCAGG TCAAACTCGA GGAGTCTGGA CCTGAGCTGG TGAAGCCTGG GGCTTCAGTG 420
AAGATATCCT GCAAGGCTTC TGGTTACTCA TTCACTGGCT ACTACATGCA CTGGGTGAAG 480
CAAAGCCATG TAAAGAGCCT TGAGTGGATT GGACGTATTA ATCCTTACAA TGGTGCTACT 540
AGCTACAACC AGAATTTCAA GGACAAGGCC AGCTTGACTG TAGATAAGTC CTCCAGCACA 600

GCCTACATGG AGCTCCACAG CCTGACATCT GAGGACTCTG CAGTCTATTA CTGTGCAAGA 660
GAGAATTACT ACGGTAGTAG CTACGGGTTT GCTTACTGGG GCCAAGGGAC TCTGGTCACT 720
GTCTCTGCA 729

INFORMATION FOR SEQ ID NO: 5

SEQUENCE CHARACTERISTICS:
LENGTH: 27 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

GGACATATGC TGAACACGAC AATTCTG

INFORMATION FOR SEQ ID NO: 6

SEQUENCE CHARACTERISTICS:
LENGTH: 29 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

GAAAAGCTTC TATTATGAAG TATTGCTCC

INFORMATION FOR SEQ ID NO: 7

SEQUENCE CHARACTERISTICS:
LENGTH: 23 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

CAGGTSMARC TCGAGSAGTC WGG

INFORMATION FOR SEQ ID NO: 8

SEQUENCE CHARACTERISTICS:
LENGTH: 32 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

AAGCTTCATG AGGAGACGGT GACCGTGGTC CC

INFORMATION FOR SEQ ID NO: 9

SEQUENCE CHARACTERISTICS:
LENGTH: 45 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

ACAGTCATAA TGTCCCATAT GGAYATYCWG MTGACMCAGT CTCCA

INFORMATION FOR SEQ ID NO: 10

SEQUENCE CHARACTERISTICS:
LENGTH: 32 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

GCATCGTCGA CTTTGAGCTC CAGCTTGGTC CC

INFORMATION FOR SEQ ID NO: 11

SEQUENCE CHARACTERISTICS:
LENGTH: 87 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

CGATTAGTAA GGAGGTTTCA TATGTCGACA AATCCTCAGG ATCTGGCTCC GAATCCAAAA 60
GCACGCAGGT CAAACTCGAG AAGCTTG 87

INFORMATION FOR SEQ ID NO: 12

SEQUENCE CHARACTERISTICS:
LENGTH: 89 base pairs
TYPE: nucleic acids
STRANDEDNESS: single
TOPOLOGY: linear
HYPOTHETICAL: yes
SEQUENCE DESCRIPTION:

GATCCAAGCT TCTCGAGTTT GACCTGCGTG CTTTTGGATT CGGAGCCAGA TCCTGAGGAT 60

TTGTCGACAT ATGAAACCTC CTTACTAAT 89

AS/hj3

Claims

1. A polypeptide, which specifically binds to the γ-chain of the interleukin-2 receptor and has the activity of blocking the interleukin 2 response.

2. A polypeptide according to Claim 1, which specifically binds to the γ-chain of the human interleukin-2 receptor.

3. The polypeptide according to Claim 1 or Claim 2, which is a monoclonal antibody.

4. The polypeptide according to any of the Claims 1 to 3, which is a mouse monoclonal antibody.

5. The polypeptide according to any of the preceeding Claims, which is a monoclonal antibody produced by the cells GP-2 (FERM BP-4641), GP-4 (FERM BP-4640), TUGh4 (FERM BP-4642), TUGh5 (FERM BP-4643) or AG14 (FERM BP-4648).

6. A hybridoma capable of producing a monoclonal antibody according to any of the Claims 3 to 5.

7. The polypeptide according to any of the Claims 1 to 5, which lacks the constant region of a monoclonal antibody.

8. The polypeptide according to any of the Claims 1 to 5, which is selected from the group consisting of (a) the following amino acid sequence

```
Met Asp Ile Leu Leu Thr Gln Ser Pro Ser Ile Met Ser Ala Ser Leu
 1               5                  10                  15
Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser
            20                  25                  30
Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu
        35                  40                  45
Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe
    50                  55                  60
Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met
65                  70                  75                  80
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Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser
                85                  90                  95
Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Val Asp Lys
            100                 105                 110
Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln Val Lys Leu Glu
        115                 120                 125
Glu Ser Gly Ser Glu Leu Val Arg Pro Gly Ala Ser Val Lys Leu Ser
    130                 135                 140
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met His Trp Val
145                 150                 155                 160
Lys Gln Arg His Gly Gln Gly Leu Glu Trp Ile Gly Asn Ile Tyr Pro
                165                 170                 175
Gly Ser Gly Ser Thr Asn Tyr Asp Glu Lys Phe Lys Ser Lys Ala Thr
            180                 185                 190
Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr Met His Leu Ser Ser
        195                 200                 205
Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Thr Arg Ser Ser Arg
    210                 215                 220
Asn Trp Val Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
225                 230                 235                 240
Thr Val Ser Ser
```

(b) a polypeptide, which in respect to (a) is deficient in one or more amino acids;

(c) a polypeptide, in which in respect to (a) or (b) one or more amino acids are replaced;

(d) a fusion peptide comprising a polypeptide according to (a), (b) or (c), in which the additively connected amino acids do not interfere with the activity of the polypeptide or which may be easily removed;

(e) a polypeptide, which in respect to (a), (b), (c) or (d) lacks the N-terminal Met;

(f) a polypeptide, in which in respect to (a), (b), (c), (d) or (e) one ore more amino acids are chemically modified.

9. The polypeptide according to any of the Claims 1 to 5, which is selected from the group consisting of (a) the following amino acid sequence

```
Met Asp Ile Leu Leu Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val
 1               5                  10                  15
Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn
            20                  25                  30
Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu
        35                  40                  45
Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser
    50                  55                  60
```

```
Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln
 65                  70                  75                  80
Pro Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro
                 85                  90                  95
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Val Asp Lys Ser
            100                 105                 110
Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln Val Lys Leu Glu Glu
        115                 120                 125
Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
    130                 135                 140
Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Tyr Met His Trp Val Lys
145                 150                 155                 160
Gln Ser His Val Lys Ser Leu Glu Trp Ile Gly Arg Ile Asn Pro Tyr
                165                 170                 175
Asn Gly Ala Thr Ser Tyr Asn Gln Asn Phe Lys Asp Lys Ala Ser Leu
            180                 185                 190
Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu His Ser Leu
        195                 200                 205
Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Tyr
    210                 215                 220
Gly Ser Ser Tyr Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
225                 230                 235                 240
Val Ser Ala
```

(b) a polypeptide, which in respect to (a) is deficient in one or more amino acids;
(c) a polypeptide, in which in respect to (a) or (b) one or more amino acids are replaced;
(d) a fusion peptide comprising a polypeptide according to (a), (b) or (c) in which the additively connected amino acids do not interfere with the activity of the polypeptide or which may be easily removed;
(e) a polypeptide, which in respect to (a), (b), (c) or (d) lacks the N-terminal Met;
(f) a polypeptide in which in respect to (a), (b), (c), (d) or (e) one ore more amino acids are chemically modified.

10. The polypeptide according to Claim 8 or 9, in which the chemical modification has been effected by acetylating, amidating or with polyethylene glycol(s).

11. The polypeptide according to any of the Claims 3 to 5, in which the constant region of the antibody has been replaced by a constant region of another species.

12. The polypeptide according to Claim 11, in which the constant region of a mouse monoclonal antibody has been replaced by a constant region of a human antibody.

13. A pharmaceutical composition comprising a polypeptide according to any of the Claims 1 to 5 and 7 to 12.

14. The pharmaceutical composition according to Claim 13, which additionally contains other active ingredients.

15. The pharmaceutical composition according to claim 14, in which the additional active ingredients are
(i) an anti-human interleukin-2 receptor α-chain antibody and/or the variable region thereof or
(ii) an anti-human interleukin-2 receptor γ-chain antibody and/or the variable region thereof and/or
(iii) derivatives of (i) and/or (ii) which are selected from the group consisiting of
(b) a polypeptide, which in respect to (a) is deficient in one or more amino acids;
(c) a polypeptide, in which in respect to (a) or (b) one or more amino acids are replaced;
(d) a fusion peptide comprising a polypeptide according to (a), (b) or (c) in which the additively connected amino acids do not interfere with the activity of the polypeptide or which may be easily removed;

(e) a polypeptide, which in respect to (a), (b), (c) or (d) lacks the N-terminal Met;
(f) a polypeptide in which in respect to (a), (b), (c), (d) or (e) one ore more amino acids are chemically modified.

16. A DNA coding for a polypeptide according to any of the Claims 1 to 5 and 7 to 12.

17. The DNA according to Claim 16, which has a nucleotide sequence selected from the group consisting of

(a) a DNA-sequence having the following nucleotide sequence

```
ATG GAC ATC CTG CTG ACC CAG TCT CCA TCA ATC ATG TCT GCA TCT CTA   48
GGG GAA CGG GTC ACC ATG ACC TGC ACT GCC AGC TCA AGT GTA AGT TCC   96
AGT TAC TTG CAC TGG TAC CAG CAG AAG CCA GGA TCC TCC CCC AAA CTC  144
TGG ATT TAT AGC ACA TCC AAC CTG GCT TCT GGA GTC CCA GCT CGC TTC  192
AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC AGC ATG  240
GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAC CAG TAT CAT CGT TCC  288
CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTC AAA GTC GAC AAA  336
TCC TCA GGA TCT GGC TCC GAA TCC AAA AGC ACG CAG GTC AAA CTC GAG  384
GAG TCT GGA TCT GAG CTG GTG AGG CCT GGA GCT TCA GTG AAG CTG TCC  432
TGC AAG GCT TCT GGC TAC ACA TTC ACC AGC TAC TGG ATG CAC TGG GTG  480
AAG CAG AGG CAT GGA CAA GGC CTT GAG TGG ATT GGA AAT ATT TAT CCT  528
GGT AGT GGT AGT ACT AAC TAC GAT GAG AAG TTC AAG AGC AAG GCC ACA  576
CTG ACT GTA GAC ACA TCC TCC AGC ACA GCC TAC ATG CAC CTC AGC AGC  624
CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT ACA AGA AGC AGC CGG  672
AAC TGG GTC TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC  720
ACC GTC TCC TCA                                                 732
```

(b) a DNA-sequence, which in respect to (a) is deficient in one or more nucleotides;
(c) a DNA-sequence, in which in respect to (a) one or more nucleotides are replaced;
(d) a DNA-sequence, to which in respect to (a) one or more nucleotides are added.

18. The DNA according to Claim 16, which has a nucleotide sequence selected from the group consisting of

(a) a DNA-sequence having the following nucleotide sequence

```
ATG GAT ATT CTG CTG ACA CAG TCT CCA GCC TCC CTA TCT GCA TCT GTG   48
GGA GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GGG AAT ATT CAC AAT   96
TAT TTA GCA TGG TAT CAG CAG AAA CAG GGA AAA TCT CCT CAG CTC CTG  144
GTC TAT AAT GCA AAA ACC TTA GCA GAT GGT GTG CCA TCA AGG TTC AGT  192
GGC AGT GGA TCA GGA ACA CAA TAT TCT CTC AAG ATC AAC AGC CTG CAG  240
CCT GAA GAT TTT GGG AGT TAT TAC TGT CAA CAT TTT TGG AGT ACT CCG  288
TGG ACG TTC GGT GGA GGG ACC AAG CTG GAG CTC AAA GTC GAG AAA TCC  336
TCA GGA TCT GGC TCC GAA TCC AAA AGC ACG CAG GTC AAA CTC GAG GAG  384
TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT TCA GTG AAG ATA TCC TGC  432
AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC TAC ATG CAC TGG GTG AAG  480
CAA AGC CAT GTA AAG AGC CTT GAG TGG ATT GGA CGT ATT AAT CCT TAC  528
AAT GGT GCT ACT AGC TAC AAC CAG AAT TTC AAG GAC AAG GCC AGC TTG  576
ACT GTA GAT AAG TCC TCC AGC ACA GCC TAC ATG GAG CTC CAC AGC CTG  624
ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA GAG AAT TAC TAC  672
GGT AGT AGC TAC GGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT  720
GTC TCT GCA                                                     729
```

(b) a DNA-sequence, which in respect to (a) is deficient in one or more nucleotides;
(c) a DNA-sequence, in which in respect to (a) one or more nucleotides are replaced;

(d) a DNA-sequence, to which in respect to (a) one or more nucleotides are added.

19. A recombinant DNA comprising a DNA according to any of the Claims 16 to 18.

20. A vector comprising a DNA according to any of the Claims 16 to 19.

21. A vector according to Claim 20, which is a plasmid.

22. A cell transformed with a DNA-sequence according to any of the Claims 16 to 21.

23. A cell according to Claim 22, which is a procaryote.

24. A cell according to Claim 22 or Claim 23, which is Escherichia coli.

25. A cell according to Claim 24, which is FERM BP-4636 or FERM BP-4637.

26. A cell according to Claim 22 which is an eucaryote.

27. A method for producing a polypeptide according to any of the Claims 1 to 5 and 7 to 12, which comprises
incubating a transformant according to any of Claim 22 to 26 and
collecting the polypeptide.

FIG. 1

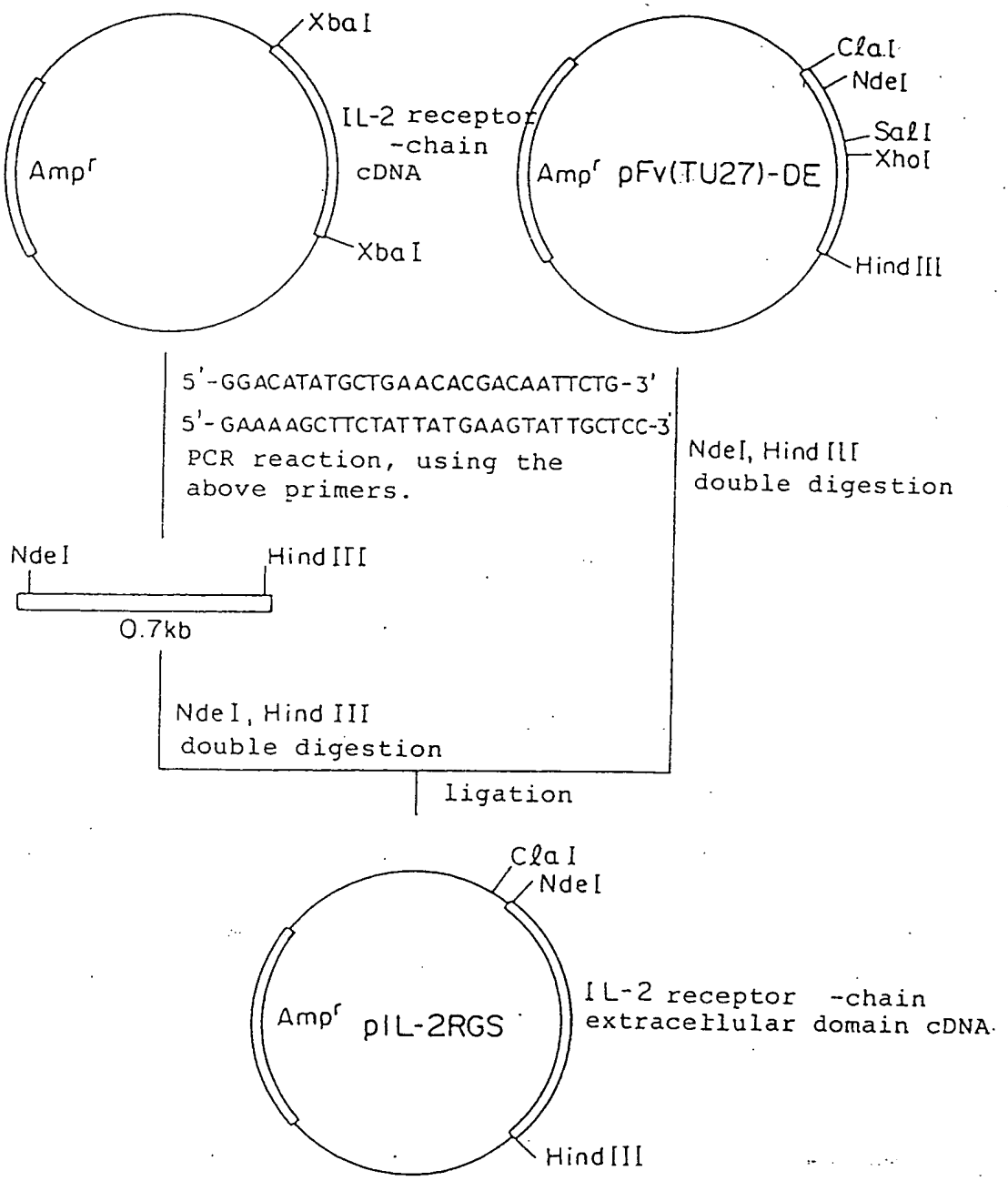

XbaI
IL-2 receptor -chain cDNA
XbaI
Ampr
ClaI
NdeI
SalI
XhoI
Ampr pFv(TU27)-DE
HindIII
5'-GGACATATGCTGAACACGACAATTCTG-3'
5'-GAAAAGCTTCTATTATGAAGTATTGCTCC-3'
PCR reaction, using the above primers.
NdeI, HindIII double digestion
NdeI
HindIII
0.7kb
NdeI, HindIII double digestion
ligation
ClaI
NdeI
Ampr pIL-2RGS
IL-2 receptor -chain extracellular domain cDNA
HindIII

FIG. 2

(a) 5'-CAGGTGAAACTCGAGCAGTCAGG-3'

CC G G T

(b) 5'-AAGCTTCATGAGGAGACGGTGACCGTGGTCCC-3'

(c) 5'-ACAGTCATAATGTCCCATATGGACATTCTGCTGACACAGTCTCCA-3'

T C A A C

(d) 5'-GCATCGTCGACTTTGAGCTCCAGCTTGGTCCC-3'

(a) is H-chain 5'-side primer; (b) is H-chain 3'-side primer;
(c) is L-chain 5'-side primer; (d) is L-chain 3'-side primer.
(a) and (c) each have two different bases at five positions.
The underlined part in (b) corresponds to the termination codon.

FIG. 3

<ClaI> <NdeI><SalI>

5'-CGATTAGTAAGGAGGTTTCATATGTCGACAAATCCTCAGGATCTGGCTCCGAATCCAAAA

3'- TAATCATTCCTCCAAAGTATACAGCTGTTTAGGAGTCCTAGACCGAGGCTTAGGTTTT

<XhoI><HindIII><BamHI>

GCACGCAGGTCAAACTCGAGAAGCTTG =3'

CGTGCGTCCAGTTTGAGCTCTTCGAACCTAG-5'

FIG. 4

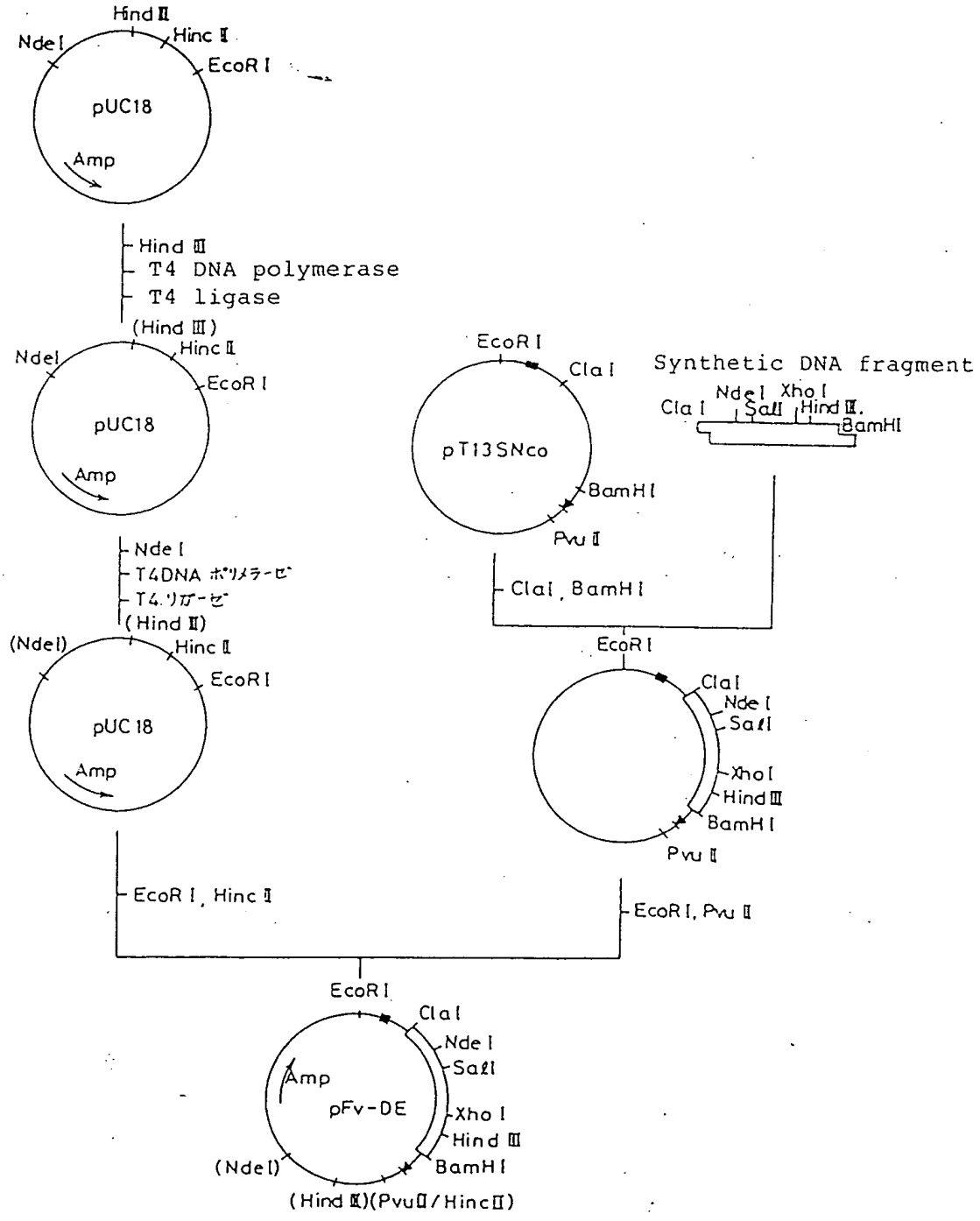

Hind Ⅲ
Nde I
Hinc Ⅱ
EcoR I
pUC18
Amp
Hind Ⅲ
T4 DNA polymerase
T4 ligase
(Hind Ⅲ)
Nde I
Hinc Ⅱ
EcoR I
pUC18
Amp
Nde I
T4DNA ポリメラーゼ
T4 リガーゼ
(Hind Ⅱ)
(Nde I)
Hinc Ⅱ
EcoR I
pUC 18
Amp
EcoR I
Cla I
pT13SNco
BamH I
Pvu Ⅱ
Synthetic DNA fragment
Cla I
Nde I
Xho I
Sal I
Hind Ⅲ
BamH I
Cla I, BamH I
EcoR I
Cla I
Nde I
Sal I
Xho I
Hind Ⅲ
BamH I
Pvu Ⅱ
EcoR I, Hinc Ⅱ
EcoR I, Pvu Ⅱ
EcoR I
Cla I
Nde I
Sal I
Amp
pFv-DE
Xho I
Hind Ⅲ
(Nde I)
BamH I
(Hind Ⅲ)(Pvu Ⅱ/Hinc Ⅱ)

FIG. 5

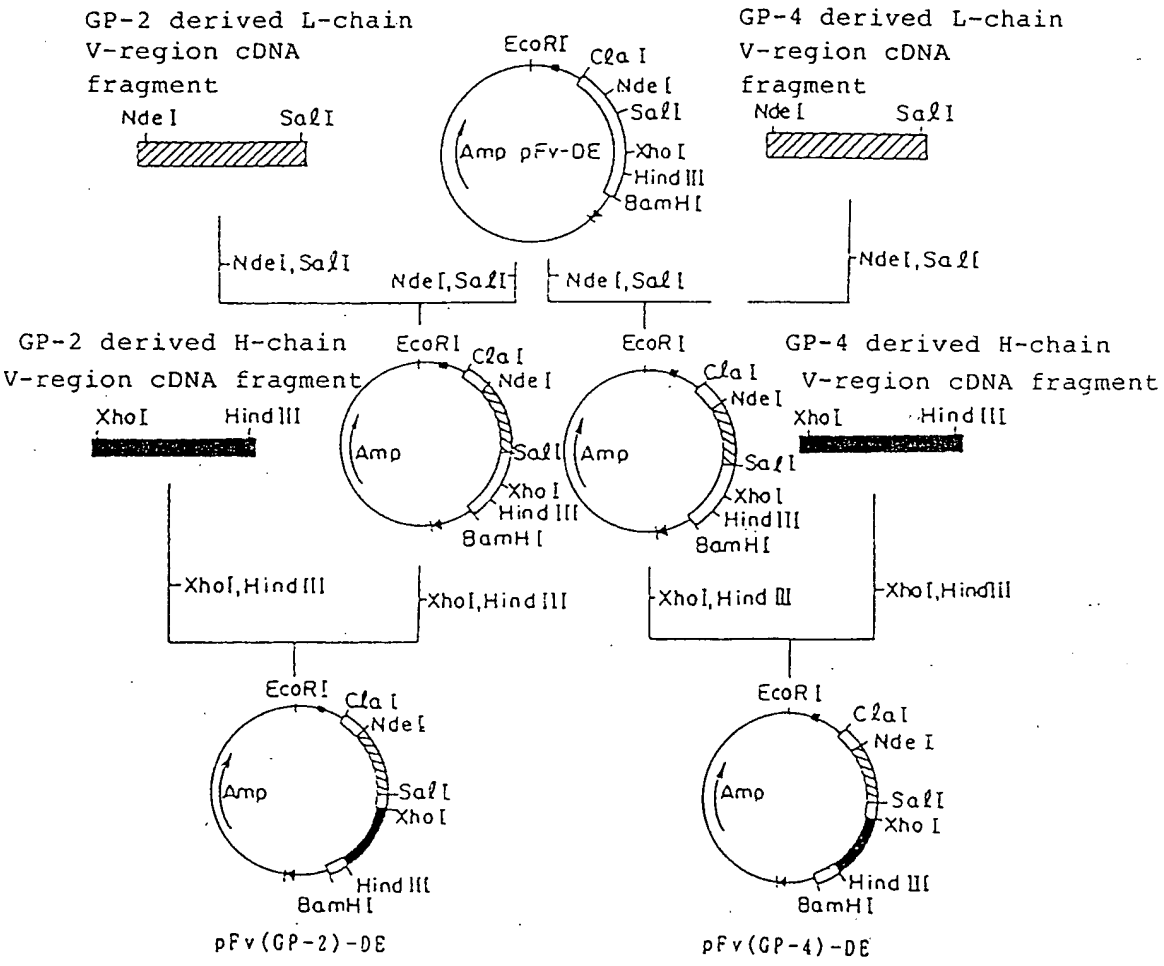
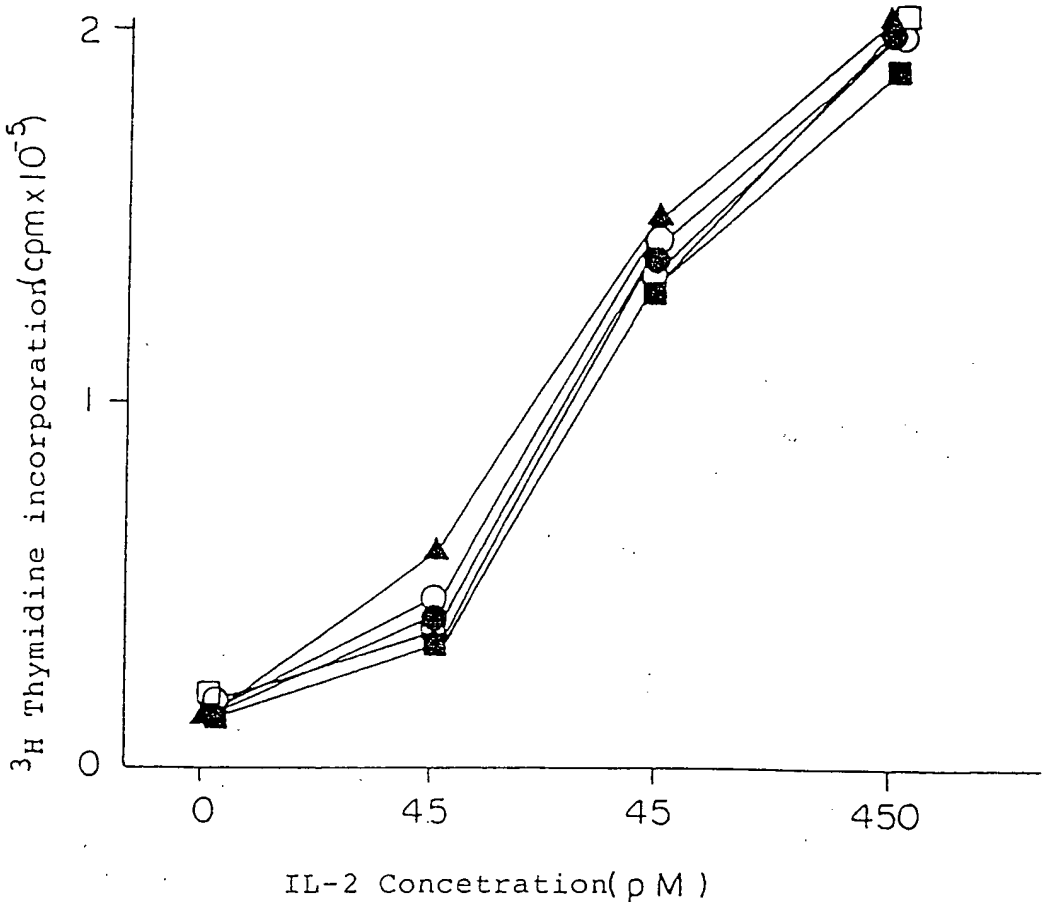
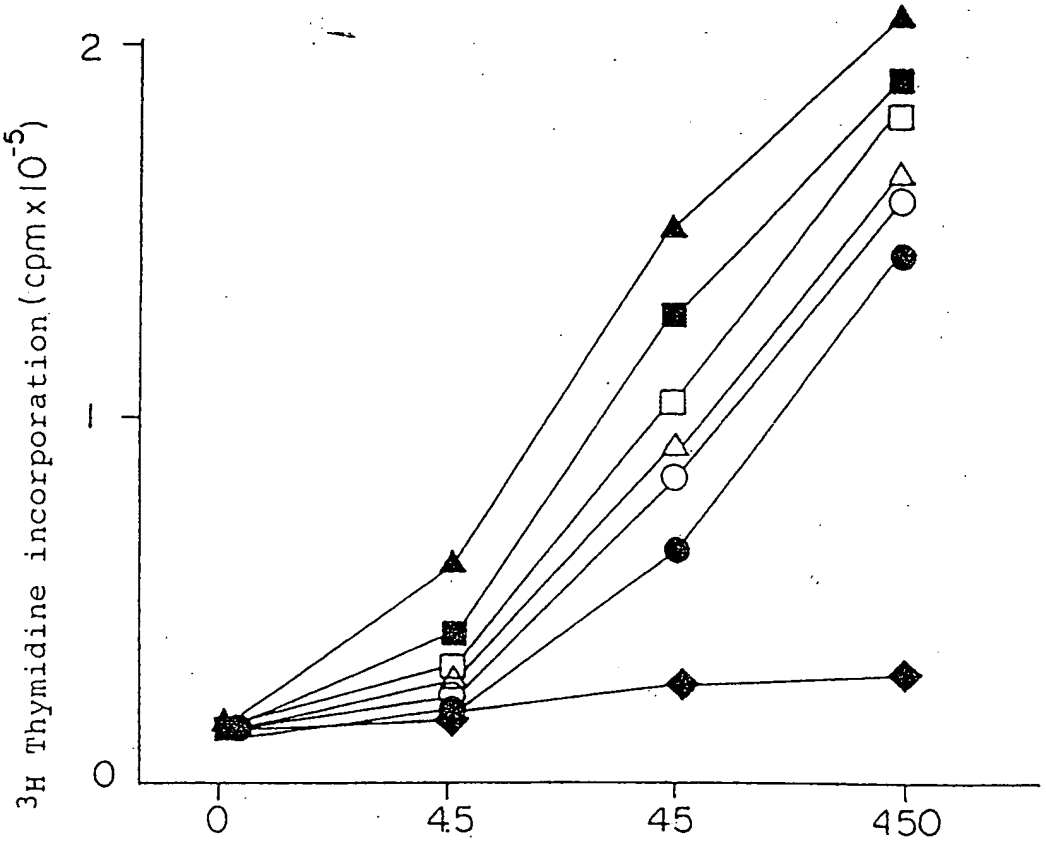

GP-2 derived L-chain V-region cDNA fragment
NdeI
SalI
EcoRI
ClaI
NdeI
SalI
XhoI
HindIII
BamHI
Amp pFv-DE
NdeI, SalI
GP-4 derived L-chain V-region cDNA fragment
NdeI
SalI
NdeI, SalI
GP-2 derived H-chain V-region cDNA fragment
XhoI
HindIII
EcoRI
ClaI
NdeI
SalI
XhoI
HindIII
BamHI
Amp
XhoI, HindIII
GP-4 derived H-chain V-region cDNA fragment
XhoI
HindIII
EcoRI
ClaI
NdeI
SalI
XhoI
HindIII
BamHI
Amp
XhoI, HindIII
pFv(GP-2)-DE
pFv(GP-4)-DE

FIG. 6



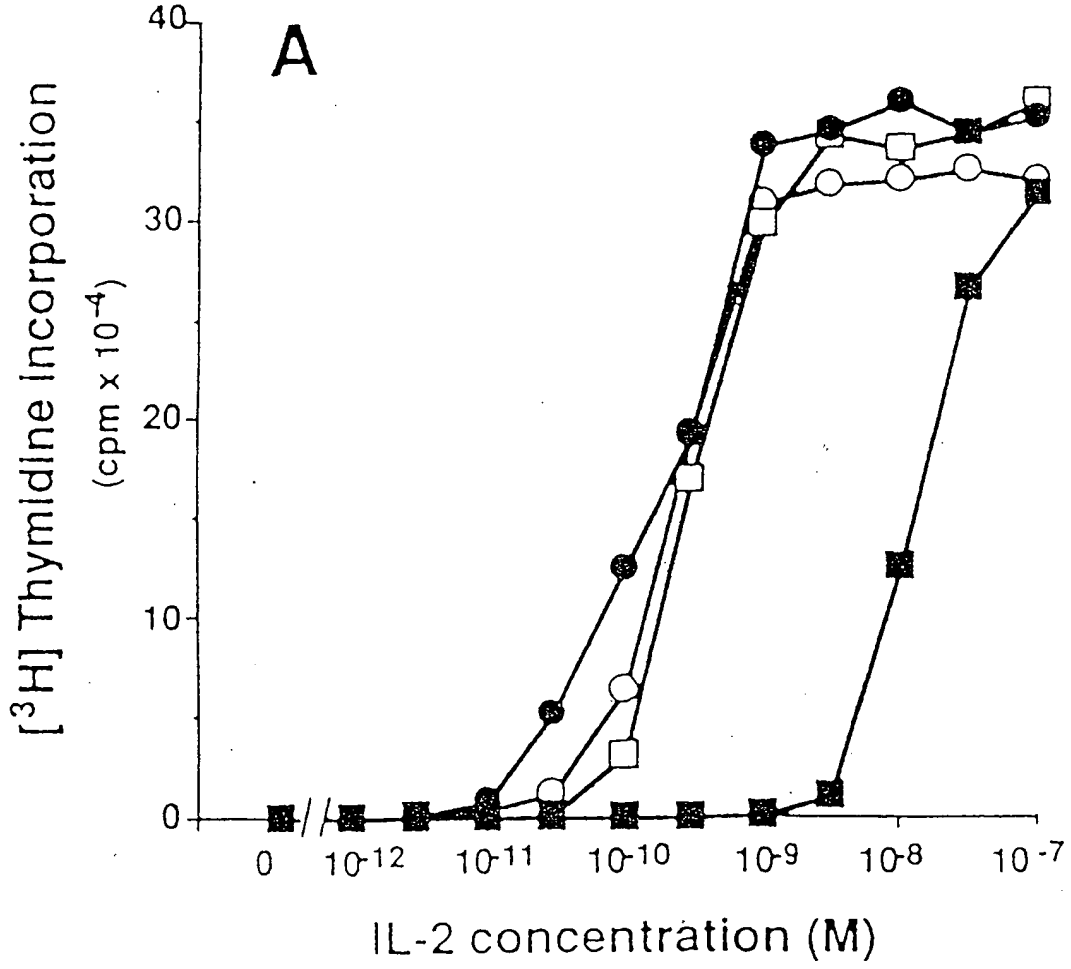
▲ ; Control antibody added.
■ ; GP-2 added.
□ ; Fv(GP-2) added.
● ; GP-4 added.
○ ; Fv(GP-4) added.

FIG. 7



▲ ; Control antibody added.
■ ; GP-2 added.
□ ; Anti-IL-2R -chain antibody added.
△ ; Anti-IL-2R -chain antibody + GP-2 added.
○ ; Anti-IL-2R -chain antibody added.
● ; Anti-IL-2R -chain antibody + GP-2 added.
◆ ; Anti-IL-2R -chain antibody + anti-IL-2R -chain antibody + GP-2 added.

FIG. 8


A
40
30
20
10
0
[3H] Thymidine Incorporation
(cpm x 10-4)
0
10-12
10-11
10-10
10-9
10-8
10-7
IL-2 concentration (M)